FORM PTO-1390 (REV 10-2000)	US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		7024491PUR92
		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/700869

INTERNATIONAL APPLICATION NO. PCT/US99/10821	INTERNATIONAL FILING DATE May 18, 1999	PRIORITY DATE CLAIMED May 18, 1998

TITLE OF INVENTION
CALCIUM CHANNEL REGULATORS

APPLICANT(S) FOR DO/EO/US
William L. PAK; Chenjian LI; Chaoxian GENG

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. [X] This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).

4. [X] The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [X] is not required, as the application was filed in the United States Receiving Ofice (RO/US).

6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).

7. [X] Amendments to the claims of the International Application under PCT Article 19(35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.

8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C 371(c)(3)).

9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). unsigned

10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [X] Other items or information:
   A. PCT Request
   B. Resp. to International Search Rpt
   C. International Publication
   D. PCT/IB/304 308, 332
   E. Notif of Receipt of Demand
   F. IPER rpt
   G. Corrected typo in Abstract as suggested by IPEA

Express Mail Label No.:EL414478366US
Date of Deposit: _20 November 2000_
I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.
Linda S. W. Coman
Signature of person mailing paper or fee

U.S. APPLICATION NO. (if known, See 37 CFR 1.5) 09/700869	INTERNATIONAL APPLICATION NO. PCT/US99/10821	ATTORNEY'S DOCKET NUMBER 7024491PUR92

17. [X] The following fees are submitted:

	CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

Neither international preliminary examination fee (37 CFR 1.482)
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO
and International Search Report not prepared by the EPO or JPO · · · · · · · · · $1000.00

International preliminary examination fee (37 CFR 1.482) not paid to
USPTO but International Search Report prepared by the EPO or JPO· · · · · · · $860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but
international search fee (37 CFR 1.445(a)(2)) paid to USPTO · · · · · · · · · · $710.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)
but all claims did not satisfy provisions of PCT Article 33(1)-(4) · · · · · · · · .$690.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)
and all claims satisfied provisions of PCT Article 33(1)-(4) · · · · · · · · · · · · .$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT = $ 100⁰⁰

Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30
months from the earliest claimed priority date (37 CFR 1.492(e)). $

CLAIMS .	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	43 - 20 =	23	X $18.00	$ 414⁰⁰	
Independent claims	14 - 3 =	11	X $80.00	$ 880⁰⁰	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$	

TOTAL OF ABOVE CALCULATIONS = $ 1394⁰⁰

[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above
are reduced by 1/2. $

SUBTOTAL = $ 1394⁰⁰

Processing fee of **$130.00** for furnishing the English translation later than [ ]20 [ ]30
months from the earliest claimed priority date (37 CFR 1.492(f)). + $

TOTAL NATIONAL FEE = $ 1394⁰⁰

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property + $

TOTAL FEES ENCLOSED = $ 1394⁰⁰

	Amount to be refunded:	$
	charged:	$

a. [X] A check in the amount of $ 1394⁰⁰ to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees.
A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
overpayment to Deposit Account No. 23-3030 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR
1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
SCHWARTZ, Jason J.
WOODARD, EMHARDT, NAUGHTON, MORIARTY & MCNETT
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SIGNATURE:

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#43,910

REGISTRATION NUMBER

IN THE INTERNATIONAL BUREAU OF WIPO
526 Rec'd PCT/PTO 2 0 NOV 2000

In re PCT application of PURDUE RESEARCH FOUNDATION, etal	)) Authorized Officer:) Gabriele Elisabeth Bugaisky)
International Application Number PCT/US99/10821	)) Mailing Date) 02 November 1999
International Filing Date 18 May 1999	)) Agent's File) Reference:
Title of Invention CALCIUM CHANNEL REGULATORS	) 7024381Pur92)

RESPONSE TO THE INTERNATIONAL SEARCH REPORT

The International Bureau
WIPO
34, chemin des Colombettes
1211 Geneva 20
Switzerland

Dear Sir/Madam:

In response to the International Search Report mailed 09 September 1999, regarding the above-referenced PCT Patent Application, Applicant does not wish to make any amendments at this time.

Respectfully submitted,

By _____
Thomas Q. Henry #28,309
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317-634-3456

#65927

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FEB 2 0 2001
PATENT & TRADEMARK OFFICE

as:7024-491:117787

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	Before the Examiner
William L. Pak et al.	)	(Unassigned)
	)	
Serial No. 09/700,869	)	
	)	
Filing Date: November 20, 2000	)	February 14, 2001
	)	
CALCIUM CHANNEL REGULATORS	)	

SCANNED, #___

SUBMITTAL OF DECLARATION / POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Although a Notice of Missing Parts has not yet been received by Applicant, pursuant to

35 U.S.C. § 371(c)(4), Applicant files herewith their Declaration/Power of Attorney which was

previously filed unsigned. A check in the amount of $130 is enclosed in accordance with 37

C.F.R. § 1.492(e). Should any other fee be required, please charge such fee to Deposit Account

No. 23-3030, but not to include any payment of issue fees.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on:

February 14, 2001
(Date of Deposit)

Jason J. Schwartz
Name of Registered Representative
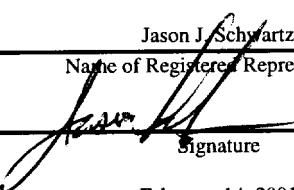
Signature

February 14, 2001
Date of Signature

Respectfully Submitted,
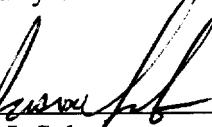
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CALCIUM CHANNEL REGULATORS

CROSS-REFERENCE TO RELATED APPLICATIONS

5   The present application claims the benefit of
U.S. Provisional Patent Application Serial Number
60/087,368, filed on May 18, 1998, and U.S. Provisional
Patent Application Serial Number 60/098,072, filed on
August 27, 1998, both of which are hereby incorporated
10   by reference in their entirety.

BACKGROUND OF THE INVENTION

In many types of excitable and nonexcitable cells,
Ca^{2+} is both a critical molecule for homeostasis and an
15   intracellular signaling molecule in many physiological
processes such as muscle contraction, glandular
secretion, transcriptional activation, and
neurotransmitter release [Berridge, M.J. (1993) *Nature*
361: 315-325; Berridge, M.J. (1995) *Biochem. J.*, 312:1-
20   11; Clapham, D.E. (1995) *Cell* 80:259-268; Clapham, D.E.
(1996) *Neuron* 16:1069-1072]. Mobilization of Ca^{2+} is
also involved in the immune response, such as
autoimmune diseases and generation of an immune
response after organ transplantation. Furthermore, a
25   growing body of evidence suggests that neuronal
degeneration diseases such as Alzheimer's is caused by
excessive Ca^{2+} mobilization. These physiological
processes are controlled by regulation of the cytosolic
free Ca^{2+} concentration ($[Ca^{2+}]_i$). In resting cells,
30   the cytosolic $[Ca^{2+}]_i$ is maintained at about 10-100 nM,

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but during stimulation the cytosolic $[Ca^{2+}]_i$ can rise rapidly to micromolar ranges.

The widely used signal transduction pathway, the receptor-based, G protein-coupled, PLC-IP3 cascade,

5   also uses Ca^{2+} as a key signaling molecule. In excitable cells such as muscle cells, Purkinje neurons and *Drosophila* photoreceptor cells, as well as in nonexcitable cells such as mast cells and lymphocytes, extracellular stimuli activate receptors on the cell

10   membrane, which in turn activate receptor-coupled G proteins. The activated G protein then activates phospholipase C to hydrolyze PIP2 to IP3 and DAG. While DAG activates phosphokinases, IP3 binds to IP3 receptors, which are ligand-gated Ca^{2+} channels on the

15   surface of intracellular Ca^{2+} stores, and induces Ca^{2+} release from these stores. The Ca^{2+} release from intracellular stores triggers, through unknown molecules and mechanisms, Ca^{2+} influx from the extracellular space into the cell via Ca^{2+} selective

20   channels on the plasma membrane (reviewed by Berridge, 1995; Clapham, 1996, both cited above).

Putney, in *Cell Calcium* 11:611-624 (1990), proposed that activation of the Ca^{2+} channel on the plasma membrane is dependent on Ca^{2+} release from the

25   intracellular stores, and named these specific types of Ca^{2+} channels on the plasma membrane "capacitative Ca^{2+} channels". In recent years, "capacitative Ca^{2+}

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channels" has been renamed "store-operated Ca^{2+} channels (SOC)" because, unlike the capacitors in electronic circuitry, the Ca^{2+} channels on the cell membranes actually pass ions through them. Cells

5 throughout the animal kingdom, as well as some bacterial, fungal and plant cells, have one or more types of calcium channels.

Although physiological and pharmacological studies identified the SOCs as a unique and important class of

10 Ca^{2+} channels, no actual genes or proteins had been identified until the *Drosophila trp* gene was cloned and subsequently studied [Montell, C. and Rubin, G.M. (1989) *Neuron* 2:1313-1323; Wong, F.E.L. et al. (1989) *Neuron* 3:81-94; Hardie, R.C. and Minke, B. (1992)

15 *Neuron* 8:643-651; Vaca, L. et al. (1994) *Am.J. Physiol.* 267:C1510-C1505]. Several lines of research have subsequently confirmed that the Drosophila Trp protein is a member of the SOCs. Since identification of the trp gene in Drosophila, several human and mouse

20 homologs have been cloned [Wes, P.D. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:9652-9656; Zhu, X. et al. (1995) *FEBS Letter* 373:193-198; Zitt, C. et al.(1996) *Neuron* 16:1189-1196]. Expression of the human Trp in COS cells increases store-operated calcium entry

25 (SOCE), and expression of portions of mouse *trp* homologs in antisense orientation in murine L cells suppressed SOCE [Zhu, X. et al. (1995) above].

Further studies have determined that another protein, InaD, binds to the *Drosophila* Trp protein.

30 InaD is a soluble protein with PDZ domains which are

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known to be important for protein/protein interaction
and anchoring ion channels [Kim, E. et al. (1995)
Nature 378:85-88; Kim, E. et al. (1996) *Neuron* 17:103-
113; Kormau, H.C. et al. (1995) *Science* 269:1737-1740].

5   InaD has been shown by co-immunoprecipitation and gel-
overlay assays to bind physically to the trp protein
(Shieh, B. and Zhu, M. (1996), *Neuron* 16:991-998;
Huber, A. et al. (1996) *EMBO* 15(24);7036-7045]. It now
appears that InaD forms the scaffold for a

10   multimolecular signaling complex that includes the TRP
protein. [Chevesich, J. et al. (1997) *Neuron* 18:95-
105; Tsunoda, S. et al. (1997) *Nature* 388:243-249].

InaC has been identified as an eye-specific
protein kinase C (Smith, D.P. et al. (1991) *Science*

15   254:1478-1484). InaC binds to InaD, suggesting that
InaD could be one of the substrates of InaC-mediated
phophorylation (Huber et al. (1996) above).

Although some information regarding regulation of
calcium ion influx into a cell is known in Drosophila

20   and higher eukaryotes, such as mice and humans,
identification of other proteins involved in Ca^{2+}
mobilization would increase the understanding of how
calcium channels are regulated. Identification of
proteins involved in calcium channel regulation in

25   lower eukaryotes can lead to identification of similar
proteins in higher eukaryotes, such as humans as
discussed above for the trp protein. Moreover,
identification of such proteins can lead to the
identification of substances that modulate the activity

30   of calcium channels, thus making it possible to treat
diseases that are thought to involve calcium ion

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mobilization, including Alzheimer's disease and
autoimmune diseases. There is therefore a need for
proteins and nucleic acid sequences involved in Ca^{2+}
mobilization. The present invention addresses this
5   need.

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SUMMARY OF THE INVENTION

A novel protein, InaF, that functions in regulation of calcium ion entry into a cell, has been discovered. Accordingly, in one aspect of the invention, purified InaF proteins are provided.

In yet another aspect of the invention, isolated nucleic acid molecules that encode InaF proteins are provided. The nucleic acid molecules may be incorporated into a vector to form a recombinant nucleic acid molecule. Moreover, such recombinant nucleic acid molecules may be introduced into a host cell.

In other aspects of the invention, methods of expressing InaF proteins are provided. The methods include transforming a host cell with a nucleotide sequence encoding a protein that functions in regulating calcium ion entry into a cell as provided herein, and culturing the transformed host cells under conditions effective in achieving expression of InaF proteins. The proteins may then be purified by conventional techniques.

It is an object of the invention to provide purified functional InaF proteins.

It is a further object of the invention to provide nucleotide sequences encoding functional InaF proteins.

It is a further object of the invention to provide recombinant vectors that include nucleotide sequences encoding functional InaF proteins.

It is yet another object of the invention to provide host cells containing introduced nucleotide sequences encoding functional InaF proteins.

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It is a further object of the invention to provide
nucleotide sequences encoding functional InaF proteins
and purified functional InaF proteins that may be
modified to control calcium ion entry into cells.

These and other objects and advantages of the
present invention will be apparent from the
descriptions herein.

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BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 depicts the cross scheme of single P local hopping mutagenesis for P69 and trol. Asterisks
5   indicate chromosomes into which the P element could have transposed.

FIG. 2 depicts electroretinogram (ERG) recordings from inaF mutants as discussed in Example 2. The top
10   trace is an ERG of strong allele, $inaF^{P111x}$, and the bottom trace is an ERG of weak allele, $inaF^{P112x}$. Stimulus duration was 4 seconds.

FIG. 3 depicts intracellularly recorded photoreceptor potentials as discussed in Example 2.
15   The voltage responses to 8 second light stimuli were measured in wild-type flies, trp^{P301}, and $inaF^{P111x}$.

FIG. 4 depicts intracellularly recorded receptor
20   potentials showing photoreceptor response latency as discussed in Example 2. Flies were dark-adapted for 2 minutes.

FIGS. 5A-B are views of photoreceptors obtained by
25   transmission electron microscopy as discussed in Example 3. FIG. 5A, left panel, depicts photoreceptors of wild-type flies; FIG. 5A, right panel, depicts photoreceptors of 19 day old inaF;bw;st reared light/dark; FIG. 5B depicts an enlarged view of the
30   region indicated by the arrow in FIG. 5A.

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FIG. 6 depicts the cross-scheme for remobilization of the P insertion in inaF^{P105P}, as discussed in Example 4.

5   FIG. 7 depicts a cytogenetic map of the inaF mutation as discussed in Example 5. Df(1)HA85(inaF⁻), Df(1)m259-4(inaF⁻) and Df(1)(inaF⁻) are deficiency stocks as discussed in Example 5.

10   FIG. 8 depicts a genomic Southern analysis as discussed in Example 6. Genomic DNA was purified and digested by EcoRI (lanes 1-4), BamHI (lanes 5-8), and HindIII (lanes 9-12), and loaded on a 0.7% agarose gel in the following order: wild-type (lanes 1, 5 and 9);
15   mutator 3B (lanes 2, 6 and 10); mutator 3B1-2 (jumpstarter)(lanes 3, 7 and 11); and inaF^{P105P} (lanes 4, 8 and 12). The gel blot was probed with ^{32}P-dCTP labeled pCaSpeR3.

20   FIG. 9 depicts a genomic Southern analysis as discussed in Example 6. Genomic DNA was purified and digested by EcoRI (lanes 1 and 2), BamHI (lanes 3 and 4), and HindII (lanes 5 and 6), and loaded on a 0.7% agarose gel in the following order: mutator 3B (lanes
25   1, 3 and 5), and inaF^{P105P} (lanes 2, 4 and 6).

FIG. 10 depicts a polytene chromosome after an in situ hybridization procedure performed as described in Example 7. The signal (arrowhead) detected on the
30   polytene chromosome was localized in the 10 C2-E3 region of the X chromosome, which was consistent with

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the results obtained by using pCaSpeR3 and fragment 4
of A23 as probes.

5   FIG. 11 depicts a Northern blot probed with cDNA
#1 insert as discussed in Example 7. The lanes were
loaded, from left to right, with polyA+ RNA from wild-
type head, wild-type body, inaF (inaF^{P105P}) head and eya
head. RP49, a ribosomal protein universally expressed
in all tissues.   .

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       FIG. 12 depicts restriction maps of inaF cDNA and
of the corresonding genomic region in the A23 clone and
three inaF mutants. The unfilled inverted triangle in
the inaF^{P105P} map identifies the P element insertion.
15   The empty spaces to the right and left of the P
insertion site in the inaF^{P106x} and inaF^{P111x} maps,
respectively, represent the deletions caused by
imprecise excision of the P element. In the cDNA map,
the broken dotted line indicates the extent of the
20   intron, and the open rectangle identifies the open
reading frame. A composite genomic map at the top
shows EcoRI sites (R) and the sizes of EcoRI fragments.

       FIG. 13 depicts a Western blot analysis of null
25   (inaF^{P106x}, trp^{P343}) and near-null (inaF^{P105P}, trp^{P301}) inaF
and trp mutants, and wild-type and revertant controls.
The seven lanes were loaded with total protein prepared
from (lanes 1-7): wild-type heads, wild-type bodies,
revertant heads, trp^{P301} heads, trp^{P343} heads, inaF^{P105P}
30   heads, and inaF^{P106X} heads.

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DESCRIPTION OF THE PREFERRED EMBODIMENTS

For the purposes of promoting an understanding of
the principles of the invention, reference will now be
5   made to preferred embodiments and specific language
will be used to describe the same. It will
nevertheless be understood that no limitation of the
scope of the invention is thereby intended, such
alterations and further modifications of the invention,
10   and such further applications of the principles of the
invention as illustrated herein, being contemplated as
would normally occur to one skilled in the art to which
the invention relates.

A novel calcium channel regulator protein, InaF,
15   has been identified in the fruit fly, *Drosophila
melanogaster*. Accordingly, the present invention
provides purified InaF protein. The invention further
provides isolated nucleic acid molecules that include
nucleotide sequences encoding functional InaF proteins.
20   Recombinant nucleic acid molecules are also provided
that include the novel inaF nucleotide sequence. The
nucleic acid molecules may be incorporated in a host
cell. In another aspect of the invention, methods of
expressing functional InaF protein are also provided.

25   In a first aspect of the invention, novel,
purified InaF proteins are provided that function in
regulating cellular influx of calcium ions. The InaF
polypeptides are substantially pure (i.e., InaF
proteins are essentially free, e.g., at least about 95%
30   free, from other proteins with which they naturally
occur). In one preferred embodiment, the amino acid

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sequence of an InaF protein, originally found in
Drosophila melanogaster, is set forth in SEQ ID:1.

Although the invention is described with reference
to *Drosophila melanogaster* amino acid sequences, it is
5   understood that the invention is not limited to the
specific amino acid sequences set forth in SEQ ID:1.
Skilled artisans will recognize that, through the
process of mutation and/or evolution, polypeptides of
different lengths and having differing constituents,
10   e.g., with amino acid insertions, substitutions,
deletions, and the like, may arise that are related to,
or sufficiently similar to, a sequence set forth herein
by virtue of amino acid sequence homology and
advantageous functionality as described herein. The
15   term "InaF protein" is used to refer generally to a
protein having the features described herein and a
preferred example includes a polypeptide having the
amino acid sequence of SEQ ID NO:1. Also included
within this definition, and in the scope of the
20   invention, are variants of the polypeptide which
function in regulating calcium ion movement into a
cell, as described herein.

It is well known that animals of a wide variety of
species commonly express and utilize homologous
25   proteins, which include the insertions, substitutions
and/or deletions discussed above, and yet which
effectively provide similar function. For example, an
amino acid sequence isolated from another species may
differ to a certain degree from the sequences set forth
30   in SEQ ID NOS:1 and 2, and yet have similar
functionality with respect to catalytic and regulatory

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function. Amino acid sequences comprising such
variations are included within the scope of the present
invention and are considered substantially or
sufficiently similar to a reference amino acid

5   sequence. Although not being limited by theory, it is
believed that the identity between amino acid sequences
that is necessary to maintain proper functionality is
related to maintenance of the tertiary structure of the
polypeptide such that specific interactive sequences

10   will be properly located and will have the desired
activity. Although it is not intended that the present
invention be limited by any theory by which it achieves
its advantageous result, it is contemplated that a
polypeptide including these interactive sequences in

15   proper spatial context will have good activity, even
where alterations exist in other portions thereof.

In this regard, an InaF protein variant is
expected to be functionally similar to that set forth
in SEQ ID NO:1, for example, if it includes amino acids

20   which are conserved among a variety of species or if it
includes non-conserved amino acids which exist at a
given location in another species that expresses a
functional InaF protein.

Another manner in which similarity may exist

25   between two amino acid sequences is where a given amino
acid of one group (such as a non-polar amino acid, an
uncharged polar amino acid, a charged polar acidic
amino acid or a charged polar basic amino acid) is
substituted with another amino acid from the same amino

30   acid group. For example, it is known that the
uncharged polar amino acid serine may commonly be

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substituted with the uncharged polar amino acid
threonine in a polypeptide without substantially
altering the functionality of the polypeptide. If one
is unsure whether a given substitution will affect the
5   functionality of the enzyme, then this may be
determined without undue experimentation using
synthetic techniques and screening assays known in the
art.

The invention therefore also encompasses amino
10   acid sequences similar to the amino acid sequences set
forth herein that have at least about 30% identity
thereto and function in regulating cellular influx of
calcium ions. Preferably, inventive amino acid
sequences have at least about 50% identity to these
15   sequences, further preferably at least about 70%
identity, more preferably at least about 80% identity
and most preferably at least about 90% identity.

Percent identity may be determined, for example,
by comparing sequence information using the advanced
20   BLAST computer program, version 2.0.8, available from
the National Institutes of Health. The BLAST program
is based on the alignment method of Karlin and
Altschul, *Proc. Natl. Acad. Sci.* USA 87:2264-68 (1990)
and as discussed in Altschul, et al., *J. Mol. Biol.*
25   215:403-10 (1990); Karlin and Altschul, *Proc. Natl.
Acad. Sci.* USA 90:5873-7 (1993); and Altschul et al.
(1997) *Nucleic Acids Res.* 25:3389-3402. Briefly, the
BLAST program defines identity as the number of
identical aligned symbols (i.e., nucleotides or amino
30   acids), divided by the total number of symbols in the
shorter of the two sequences. The program may be used

15

to determine percent identity over the entire length of
the proteins being compared. Preferred default
parameters for the BLAST program, blastp, include: (1)
description of 500; (2) Expect value of 10; (3) Karlin-

5   Altschul parameter λ = 0.270; (4) Karlin-Altschul
parameter K = 0.0470; (5) gap penalties: Existence 11,
Extension 1; (6) H value = $4.94e^{-324}$; (6) scores for
matched and mismatched amino acids found in the
BLOSUM62 matrix as described in Henikoff, S. and

10 Henikoff, J.G. (1992) *Proc. Natl. Acad. Sci. USA*
89:10915-10919; Pearson, W.R. (1995) *Prot. Sci.* 4:1145-
1160; and Henikoff, S. and Henikoff, J.G. (1993)
Proteins 17:49-61. The program also uses an SEG filter
to mask-off segments of the query sequence as

15 determined by the SEG program of Wootton and Federhen
(1993) *Computers and Chemistry* 17:149-163.

In another aspect of the invention, isolated
nucleic acid molecules, originally isolated from
Drosophila melanogaster, are provided that encode a

20 functional InaF protein that functions in regulating
calcium ion entry into cells. The nucleotide sequences
are set forth in SEQ ID NOS:1 and 2. It is preferred
that the nucleotide sequence includes nucleotides
spanning nucleotides 301 to 1036 in SEQ ID NO:1 and

25 nucleotides spanning nucleotides 528 to 1250 in SEQ ID
NO:2. It is not intended that the present invention be
limited to these exemplary nucleotide sequences, but
include sequences having substantial similarity thereto
and sequences which encode variant forms of functional

30 InaF protein as discussed above and as further
discussed below.

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The term "isolated nucleic acid," as used herein, is intended to refer to nucleic acid which is not in its native environment. For example, the nucleic acid is separated from other contaminants that naturally

5   accompany it, such as proteins, lipids and other nucleic acid sequences. The term includes nucleic acid which has been removed or purified from its naturally-occurring environment or clone library, and further includes recombinant or cloned nucleic acid isolates

10   and chemically synthesized nucleic acid.

The term "nucleotide sequence," as used herein, is intended to refer to a natural or synthetic linear and sequential array of nucleotides and/or nucleosides, including deoxyribonucleic acid and ribonucleic acid,

15   and derivatives thereof. The terms "encoding" and "coding" refer to the process by which a nucleotide sequence, through the mechanisms of transcription and translation, provides the information to a cell from which a series of amino acids can be assembled into a

20   specific amino acid sequence to produce a functional polypeptide, such as, for example, an active enzyme or other protein that has a specific function. The process of encoding a specific amino acid sequence may involve DNA sequences having one or more base changes

25   (i.e., insertions, deletions, substitutions) that do not cause a change in the encoded amino acid, or which involve base changes which may alter one or more amino acids, but do not eliminate the functional properties of the polypeptide encoded by the DNA sequence.

30   It is therefore understood that the invention encompasses more than the specific exemplary nucleotide

sequence of *inaF*. For example, nucleic acid sequences encoding variant amino acid sequences, as discussed above, are within the scope of the invention. Modifications to a sequence, such as deletions,

5   insertions, or substitutions in the sequence, which produce "silent" changes that do not substantially affect the functional properties of the resulting polypeptide molecule are expressly contemplated by the present invention. For example, it is understood that

10   alterations in a nucleotide sequence which reflect the degeneracy of the genetic code, or which result in the production of a chemically equivalent amino acid at a given site, are contemplated. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be

15   substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for

20   another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a biologically equivalent product.

Nucleotide changes which result in alteration of

25   the N-terminal and C-terminal portions of the encoded polypeptide molecule would also not generally be expected to alter the activity of the polypeptide. In some cases, it may in fact be desirable to make mutations in the sequence in order to study the effect

30   of alteration on the biological activity of the

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polypeptide. Each of the proposed modifications is
well within the routine skill in the art.

In one preferred embodiment, the nucleotide
sequence has substantial similarity to the sequence set
5   forth in SEQ ID:1 or SEQ ID:2, preferably the sequence
spanning nucleotides 314 to 1036 in SEQ ID:1 and
preferably the sequence spanning nucleotides 528 to
1250 in SEQ ID:2, and variants described herein. The
term "substantial similarity" is used herein with
10   respect to a nucleotide sequence to designate that the
nucleotide sequence has a sequence sufficiently similar
to a reference nucleotide sequence that it will
hybridize therewith under moderately stringent
conditions. This method of determining similarity is
15   well known in the art to which the invention pertains.
Briefly, moderately stringent conditions are defined in
Sambrook et al., *Molecular Cloning: A Laboratory
Manual*, 2nd ed. Vol. 1, pp. 101-104, Cold Spring Harbor
Laboratory Press (1989) as including the use of a
20   prewashing solution of 5X SSC (a sodium chloride/sodium
citrate solution), 0.5% sodium dodecyl sulfate (SDS),
1.0 mM ethylene diaminetetraacetic acid (EDTA) (pH 8.0)
and hybridization and washing conditions of 55°C, 5x
SSC. A further requirement of the inventive
25   polynucleotide is that it must encode a polypeptide
having similar functionality to the InaF protein
described herein, i.e., functioning to regulate influx
of calcium ions into cells.

In yet another embodiment, nucleotide sequences
30   having selected percent identities to specified regions
of the nucleotide sequence set forth in SEQ ID:1 are

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provided.   In one preferred form, nucleotide sequences
are provided that have at least about 60% identity,
more preferably at least about 80% identity, and most
preferably at least about 90% identity, to a nucleotide
5   sequence of substantial length within the nucleotide
sequence from nucleotides 314 to 1036 set forth in SEQ
ID:1. For example, such length may be 100, 200 or 400
nucleotides, or may be the entire sequence from
nucleotides 314 to 1036 of SEQ ID:1. A further
10   requirement is that the nucleotide sequence from
nucleotide 314 to 1036 set forth in SEQ ID:1 encodes a
protein that functions in regulating calcium entry into
cells. The percent identity may be determined, for
example, by comparing sequence information using the
15   advanced BLAST computer program, version 2.0.8., as
described above with reference to amino acid identity.
Preferred default parameters for blastn include: (1)
Karlin-Altschul parameter λ = 1.37 (gapped and
ungapped); (2) Karlin-Altschul parameter K = 0.711
20   (gapped and ungapped); (3) H = $4.94e^{-324}$ (gapped and zero
for ungapped); (4) gap penalties: Existence 5,
Extension 2; and (5) scores for matched and mismatched
nucleotides found in the blastn matrix as described in
Altschul, S.F. et al. (1997) *Nucleic Acids Res.*
25   25:3389-3402 and Zhang, J. (1997) *Genome Res.* 7:649-
656.

        A suitable DNA sequence may be obtained by cloning
techniques using cDNA libraries. For example,
Drosophila melanogaster head cDNA libraries are
30   available commercially or may be constructed using
standard methods known in the art. Suitable nucleotide

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sequences may be isolated from DNA libraries obtained
from a wide variety of species by means of nucleic acid
hybridization or polymerase chain reaction (PCR)
procedures, using as probes or primers nucleotide
5   sequences selected in accordance with the invention,
such as those set forth in SEQ ID:1, nucleotide
sequences having substantial similarity thereto, or
portions thereof.

Alternately, a suitable sequence may be made by
10   techniques which are well known in the art. For
example, nucleic acid sequences encoding a functional
InaF protein may be constructed by recombinant DNA
technology, for example, by cutting or splicing nucleic
acids using restriction enzymes and DNA ligase.
15   Furthermore, nucleic acid sequences may be constructed
using chemical synthesis, such as solid-phase
phosphoramidate technology. PCR may be used to
increase the quantity of nucleic acid produced.
Moreover, if the particular nucleic acid sequence is of
20   a length which makes chemical synthesis of the entire
length impractical, the sequence may be broken up into
smaller segments which may be synthesized and ligated
together to form the entire desired sequence by methods
known in the art.

25   In another aspect of the invention, InaF
polypeptides functioning in regulating calcium ion
entry into a cell and having the amino acid sequences
encoded by nucleotide sequences having substantial
similarity to the nucleotide sequences described above
30   are also provided.

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    In a further aspect of the invention, recombinant
nucleic acid molecules, or recombinant vectors, are
provided. In one embodiment, the nucleic acid
molecules include a nucleotide sequence encoding a
5   functional InaF protein. The nucleotide sequence has
substantial similarity, as defined above, to the
nucleotide sequence set forth in SEQ ID:1 or SEQ ID:2,
preferably the sequence spanning nucleotides 314 to
1036 in SEQ ID:1 or the identical sequence in SEQ ID:2
10   spanning nucleotides 528 to 1250. The protein produced
has the amino acid sequence set forth in SEQ ID:1, or
variants thereof as described above.

    Recombinant vectors may be constructed by
incorporating the desired nucleotide sequence within a
15   vector according to methods well known to the skilled
artisan and as described for example, in Maniatis et
al., *Molecular Cloning: A Laboratory Manual*, Cold
Springs Laboratory, Cold Springs Harbor, New York
(1982). A wide variety of vectors are known that have
20   use in the invention. For example, various plasmid and
phage vectors are known that are ideally suited for use
in the invention. For example, pGEM, pBluesript, EMBL
and λGt11 may be used in the invention. In one
embodiment, the desired recombinant vector may be
25   constructed by ligating DNA linker sequences to the 5'
and 3' ends of the desired nucleotide insert, cleaving
the insert with a restriction enzyme that specifically
recognizes sequences present in the linker sequences
and the desired vector, cleaving the vector with the
30   same restriction enzyme, mixing the cleaved vector with

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the cleaved insert and using DNA ligase to incorporate the insert into the vector as known in the art.

The vectors may include other nucleotide sequences, such as those encoding selectable markers, including those for antibiotic resistance or color selection. The vectors also preferably include a promoter nucleotide sequence. The desired nucleic acid insert is preferably operably linked to the promoter. A nucleic acid is "operably linked" to a another nucleic acid sequence, such as a promoter sequence, when it is placed in a specific functional relationship with the other nucleic acid sequence. The functional relationship between a promoter and a desired nucleic acid insert typically involves the nucleic acid and the promoter sequences being contiguous such that transcription of the nucleic acid sequence will be facilitated. Two nucleic acid sequences are further said to be operably linked if the nature of the linkage between the two sequences does not (1) result in the introduction of a frame-shift-mutation; (2) interfere with the ability of the promoter region sequence to direct the transcription of the desired nucleotide sequence, or (3) interfere with the ability of the desired nucleotide sequence to be transcribed by the promoter sequence region. Typically, the promoter element is generally upstream (i.e., at the 5' end) of the nucleic acid insert coding sequence.

A wide variety of promoters are known in the art, including cell-specific promoters, inducible promoters, and constitutive promoters. The promoters may further be selected such that they require activation by

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activating elements known in the art, so that
production of the protein encoded by the nucleic acid
sequence insert may be regulated as desired.

5   The vectors may further include other regulatory
elements, such as enhancer sequences, which cooperate
with the promoter to achieve transcription of the
nucleic acid insert coding sequence. By "enhancer" is
meant nucleotide sequence elements which can stimulate
promoter activity in a cell, such as a bacterial or
10   eukaryotic host cell.

Moreover, the vectors may include another
nucleotide sequence insert that encodes a protein that
may aid in purification of the desired protein encoded
by the desired nucleotide sequence. The additional
15   nucleotide sequence is positioned in the vector such
that a fusion, or chimeric, protein is obtained. For
example, an InaF protein may be produced having at its
C-terminal end linker amino acids, as known in the art,
joined to the other protein. The additional nucleotide
20   sequence may include, for example, the nucleotide
sequence encoding glutathione-S-transferase (GST).
After purification procedures known to the skilled
artisan, the additional amino acid sequence is cleaved
with an appropriate enzyme. For example, if the
25   additional amino acid sequence is that of GST, then
thrombin is used to separate the InaF protein from GST.
The InaF protein may then be isolated from the other
proteins, or fragments thereof, by methods known in the
art.

30   The inventive recombinant vectors may be used to
transform a host cell. Such methods include, for

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example, those described in Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Laboratory, Cold Springs Harbor, New York (1982). Once the desired nucleic acid has been introduced into the host cell,

5   the host cell may produce the inventive InaF protein, or variants thereof, as described above. Accordingly, in yet another aspect of the invention, a host cell is provided that includes the inventive recombinant vectors described above.

10   A wide variety of host cells may be used in the invention, including prokaryotic and eukaryotic host cells. Bacterial host cells such as *Escherichia coli*, HB 101 and XL-1 blue may be advantageously used in the present invention. Typical eukaryotic host cells

15   include SF9, S2, NIH 3T3 and NIH 293.

In yet another aspect of the invention, methods of producing functional InaF proteins as described above are provided. In one embodiment, the method includes providing a nucleotide sequence described above, or

20   variants thereof, that encodes a functional InaF protein that regulates calcium ion entry into cells, and introducing the nucleotide sequence into a host cell, as described above. The desired nucleotide sequence may be advantageously incorporated into a

25   vector to form a recombinant vector. The recombinant vector may then be introduced into a host cell according to known procedures in the art. Such host cells are then cultured under conditions, well known to the skilled artisan, effective to achieve expression of

30   the InaF polypeptide. The InaF polypeptide may then be purified using conventional techniques.

25

Reference will now be made to specific examples illustrating the invention described above. It is to be understood that the examples are provided to illustrate preferred embodiments and that no limitation

5   to the scope of the invention is intended thereby.

EXAMPLE 1

Generation of inaF Mutant by
P-Element Mediated Mutagenesis

10   This example shows the method by which *inaF* mutants were obtained through P-element mediated mutagenesis.

Drosophila Stocks

The first *inaF* mutant was generated through P-
15   element mediated mutagenesis, as described below, on a *white⁻* background. The actual eye color of the mutant was light orange because the P element insertion causing the *inaF* mutation contains a *mini-white⁺* gene. To eliminate eye color, the original *inaF* mutant was
20   placed in a *bw; st* background, so that *inaF; bw; st* flies would have no eye-color pigment.

In accordance with the Pak laboratory's practice of giving a P1XX number to 1st chromosome mutations and using a lower-case letter after the number to indicate
25   the method of inducing the mutations, we designated the original *inaF* as *inaF^{P105p}*, in which the lower-case p in the superscript stands for P-insertion. In the course of a remobilization experiment to be described below, 25 new *inaF* alleles were generated due to
30   imprecise excision. For these additional alleles we used a lower-case x in the superscript to indicate that

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they were induced by imprecise excision and designated
the 25 new alleles in as $inaF^{P106x}$ through $inaF^{P130x}$.

The mutator, 3B, was chosen for local hopping
mutagenesis, because it has an insertion in 3B1-2,

5   which is very close to 3A3-5 where the *P69* gene is
localized. This fly has a mutation in an eye-pigment
gene *white*, and thus originally has a white eye color
background. The actual eye color of 3B is orange,
because the fly also carries a P element, pCaspeR3,

10   which has the mini-$white^+$ gene as a marker. The shades
of eye color, from dark red to light lemon, are
dependent on where the insertions are. The eye color
is darker when the insertion is in the vicinity of a
strong enhancer, and the eye color is lighter when the

15   insertion is close to a weaker enhancer. This
location-sensitive eye color change is a very good
indication of whether the P element has been mobilized
to a new place.

The mutator, y w/P[lacW] was chosen for random

20   targeting mutagenesis. This fly carries a $white^-$
mutation, and thus has a white eye color background.
Its actual orange eye color is from the P[lacW], an
engineered P element with the plasmid rescue feature as
well as the enhancer trap capability as described in

25   Bier et al., *Genes and Development*, 3:1273-1287 (1989).

The jumpstarter, *P3629*, carries a functional
transposase gene which lacks the end inverted repeats
(delta2-3). The delta2-3 is inserted on the 3rd
chromosome, which also carries a visible dominant

30   marker Sb. This visible marker is useful in indicating
the presence or absence of the delta2-3.

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The *trol* mutation is lethal. In the *trol* stock
used in this mutagenesis, females are balanced over
FM7, and males carry, by translocation, a 2D-3C segment
of the X chromosome on the Y chromosome, which rescues
5   the *trol* lethality.

In the *C(1)RM, y w/w/Y* fly stock, females have a
special type of genome that contains two linked X
chromosomes. These two linked chromosomes will
segregate together. If a male fly is mated to *C(1)RM,*
10   *y w/Y* females, all the male offspring will carry the
same X chromosome as the P1 male.

Local hopping mutagenesis to target *trol/P69*.

The P element mediated local hopping mutagenesis
15   was undertaken with the aim of isolating lethal *trol*
alleles or viable ERG mutants (FIG. 1).   There were
three generations of crosses before the mutagenesis
result could be tested by ERG.

20   Cross I: The mutator, 3B, which carries a pCaspeR3 in

white⁻ background was used. Its eye-color is light
orange.   The jumpstarter stock carries delta2-3 on the
third chromosome, which is marked with a dominant
marker Sb. In each bottle, 20 mutator males and 20
25   jumpstarter females were combined.   Parent flies in
each bottle were transferred after 4-5 days to a new
bottle once, and then discarded. All flies were raised
at 25°C.

30   Cross II: Among the progeny of cross I, virgin
females with Sb marker were selected to mate with males

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from a FM0 containing stock.   The females carried both
the mutator on the X chromosome, and the jumpstarter on
the 3rd chromosome.   In the germ line cells of these
flies, the P element in the mutator could be mobilized
5   to new chromosomal locations because of the transposase
activity conferred by delta2-3.   In each round of the
mutagenesis, 20 bottles of cross II were set up, each
containing 20 virgin females and 20 males.   Parent
flies were transferred once to new food after 4-5 days,
10   and then discarded.   All flies were raised at 25°C.

Cross III: From the progeny of cross II, flies were
selected for remobilized pCaspeR3, by selecting for
flies with changed eye color shades (presence of
15   *white*$^+$).   Flies were also selected against Sb-marked
delta2-3, so that pCaspeR3 insertions would be
stabilized.
        Both male and female progeny of cross II were
used:
20   1) Males were single-mated to *C(1)RM, y w/Y* females
carrying an attached X chromosome to establish stable
lines.   After 7 days, male parents were scored by an
electroretinogram (ERG) as described in Example 2.   If
the ERG showed a mutant phenotype, the line was saved
25   for further study; and if the ERG were wild type, the
line was discarded.

2) Virgin females were single-mated to *trol/W$^+$Y* males.
After 7 days, parents were transferred to fresh food.
The offspring of this single-female-mating have four
30   possible genotypes, indicated as A,B,C and D in FIG. 1.

If type A flies were found, the line was discarded
because the insertion obviously was not into the *trol*
gene.   If type A flies were not found, then the P
insertion was in the *trol* gene.   The D type flies were
5   saved for further study.

Analysis

Three rounds of local hopping mutagenesis were
performed.   Cross II yielded about 2% of offspring that
showed changes of eye color, indicating that the P
10   element was mobilized to new chromosomal locations.

Approximately 2×10^4 F_2 flies were scored.

Virgin female F_2 flies with eye color changes were
single-female-mated to *trol*/W^+Y.   The offspring of this
cross were scored for complementation with *trol*.   Among
15   179 such single-female-mating lines, none was
identified as a *trol* allele.

Male F_2 flies with eye color changes were single
male mated to C(1) RM, y w/Y.   Among the offspring of
this single-male-mating, all males carried the same X
20   chromosome as the single male parent.   1-2 males of
each line were scored by ERG.   In 255 such single male
mating lines, one was identified as a new mutant and
designated as *inaF*.

EXAMPLE 2

25   **Electrophysiological Identification of *inaF* Phenotypes**

Electroretinogram

The electroretinogram (ERG) is an extracellular
measurement of the light-induced responses in the eyes.
The ERGs were recorded as described in Pak, W.L. et al.

30

Nature 222:351-354 (1969). A xenon arc lamp (Oriel)
served as the light source with an infrared filter
(7CS1-75, Corning) and Wratten neutral density filters
(Kodak) were used to modulate its intensity and

5   infrared content. In most cases, flies were raised to
5 day post-eclosion for ERG recordings. In the case of
the *P69;bw;st trp*^{CM}double mutant, however, 1 day old
flies were used because photoreceptors in the double
mutant showed massive degeneration by day 5, but no

10   visible defects in the eye structure on day 1.

Intracellular Recording

       The intracellular recording technique was
performed as described in detail by Johnson, E.C. et
al. (1986) *J. Gen. Physiol.* 88(5):651-673. Flies

15   anaesthetized with CO_2 were mounted on a glass
coverslip with myristic acid. A small portion (<10%)
of the cornea was cut off with a vibrating razor blade.
A thin layer of inert vacuum grease was applied to the
cut end to prevent desiccation of the retina.

20   Both the reference and the recording electrodes
were inserted into the eye through the cut end of the
cornea. The reference electrode was a low resistance
glass microelectrode filled with physiological saline
and was placed into the retinal layer of the eye. The

25   intracellular recording electrode (FHC Borosil 1.2 mm)
was pulled on a vertical Narashige puller, filled with
2 M KCl, and selected for resistance ranging between 30
to 100 mega ohm. The recording electrode was inserted
into the retinal layer with a Leitz micromanipulator.

30   Penetration of a photoreceptor was done by a minute

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forward movement of the electrode and a simultaneous delivery of a brief overdriving negative capacitance current to induce oscillation at the tip of the electrode. Successful penetration of a photoreceptor cell was indicated by a drop in voltage of more than 30 mV as seen on the oscilloscope and a receptor potential of more than 20 mV in response to a bright light stimulus. The preparation was dark adapted for more than 2 minutes before any further experiments.

The measured voltage was fed to a WPI preamplifier from which the signals were directed to both an oscilloscope and a digitizer (Digidata 1200, Axon Instrument). The digitized signals were filtered at 100 Hz and were recorded by Axoscope in a Pentium computer.

Analysis

In the study of inaF, all flies for ERG were 2-4 days posteclosion. The most obvious mutant phenotype of inaF revealed by ERG and intracellular recordings is that the receptor potential fails to maintain a steady-state response during light stimuli and decays rapidly toward base line (FIGS. 2 and 3).

The rate of decay was allele-dependent. Strong alleles such as $inaF^{P111x}$ caused the receptor potential to decay to base line within 4-5 seconds under bright light stimuli. Intermediate alleles such as $inaF^{P112x}$ caused slower decay, and some of them never caused complete decay to the base line even under bright light stimuli.

32

The rate of decay was also dependent on the light intensity (FIG. 2) and was faster under brighter light in all *inaF* alleles.

This receptor potential decay in *inaF* closely resembles the phenotype displayed by the *trp* mutant. When the strongest mutant alleles of the two genes, *inaF*^{P111x} and *trp*^{P301}, were compared, *inaF*^{P111x} caused a stronger mutant phenotype in speed and extent of receptor potential decay (FIG. 3). FIG. 3 also shows that the receptor potential of wild-type flies is maintained at a steady state.

Another mutant phenotype became evident when the latency between the light stimulus and the photoreceptor response was examined (FIG. 4). The latency is defined as the delay between the onset of the light stimulus and the beginning of photoreceptor depolarization. This delay is light intensity dependent and has been interpreted as the time required by the phototransduction pathway to proceed from photoconversion of rhodopsin to the opening of light-activated channels on the plasma membrane. In both *trp*^{P301} and *inaF*^{P111x}, the latency was prolonged compared to that of the wild type, and the delay was greater in *inaF*^{P111x}.

EXAMPLE 3

Effect of inaF Mutation on Retinal Degeneration

This example shows that the *inaF* photoreceptors undergo a light-dependent degeneration. Degeneration is also age-dependent and is not detectable in young (<1 week old) inaF mutants.

Transmission Electron Microscopy

     The transmission electron microscopy technique was
identical to the method described by Fan, S.S. and
Ready, D.F. (1997) *Development* 124:1497-1507. Flies
5   were microinjected with aldehyde fixative (2%
paraformaldehyde and 1.75% glutaraldehyde in 0.1 M
sodium cacodylate, pH 7.4) and dissected after 1 hour.
Fixed eyes were incubated in 1% tannic acid overnight
and transferred to 2% osmium tetroxide in 0.1 M sodium
10 cacodylate for 2 hours. After washing, the eyes were
incubated overnight in 2% uranyl acetate. After a
serial dehydration with ethanol, eyes were mounted in
Epox 812. Tissue was then sectioned using a Reichert
ultramicrotome and observed using a Philips 300
15 electron microscope.

Analysis

     Retinal degeneration was observed in *inaF* compound
eyes (FIGS. 5A-B). For better control, *inaF*^{P105p} was
put in a *bw; st* background to eliminate eye color.
20 Confocal microscopy and EM were used to examine
photoreceptor structures. The photoreceptors in
inaF^{P105p}; *bw; st* showed no detectable abnormality at 1
day posteclosion, suggesting that they had developed
normally. However, flies raised in a 12 hour-light/12
25 hour-dark cycle to 19 days posteclosion showed retinal
degeneration. Rabdomeres were absent in some
ommatidia; most of the rabdomeres were much reduced in
size; microvilli (membrane which contains rhodopsin)
were disrupted by vacuolized structures; and the base
30 of the microvilli was no longer smooth and regular.

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To test if this retinal degeneration was light

dependent, $inaF^{P105p}$; bw; st flies were raised in

complete darkness from the embryo stage to 19 days

posteclosion. EM study of these fly eyes indicated

5   that the photoreceptor structure was largely intact

(data not shown). Hence the retinal degeneration in

$inaF$ is light dependent.

EXAMPLE 4

10

Proof that the inaF Mutation is Caused by
P-Element Insertion — Remobilization of the
P insertion in $inaF^{P105p}$

15   The most direct and reliable method to demonstrate

that a mutation is in fact caused by a P element

insertion is to remobilize the P element. If the P

element is the cause of the mutation, then one would

expect two possibilities when the P insertion is

20   removed from the genome. First, if the P element is

precisely excised, then the mutated gene will be

restored, and the mutant flies revert to wild type

(thus they are called "revertants"). Second, if the

excision is imprecise and takes away some flanking DNA

25   with the remobilized P element, the flies will continue

to show a mutant phenotype.

If, however, the mutation is actually not caused

by the P insertion, but by some other defect such as a

spontaneous point mutation, then the flies with the P

30   element remobilized will always remain mutant, i.e., no

wild-type revertants will be recovered.

35

Remobilization of the P insertion in $inaF^{P105p}$

$inaF^{P105p}$ was used as a mutator and crossed to the jumpstarter, *P3269*. The design and protocol for this remobilization experiment are the same as those of

5   local hopping mutagenesis described in Example 1 above, except that white-eyed flies (indicating loss of the P element which carries the *mini-white*$^{+}$ gene) were selected among the offspring of cross II (FIG. 6). These virgin females were single mated to FMO/Y males.

10   The offspring had four possible genotypes (X- indicates an excision event):

A: X- $inaF^{P105p}$ -X/FMO; +/+; +/+

B: FMO/FMO; +/+;+/+

C: FMO/Y;+/+;+/+

15   D: X- $inaF^{P105p}$ -X/Y;+/+;+/+

Type D offspring in each single-female line were selected for ERG. If the ERG showed the *inaF* phenotype, the excision event was an imprecise one. Types A and D were saved to establish stable lines of

20   new *inaF* alleles. If the ERG showed a wild-type response, the excision event was a precise one. Types A and D were saved to establish stable lines of these revertants. These lines were used in chromosomal *in situ* hybridization with the P element as a probe to

25   confirm that the P insertion in $inaF^{P105p}$ no longer existed.

Analysis

In cross III, 260 single-female-mating lines were set up, and their offspring were scored by ERG. We obtained 126 wild-type revertants, 61 lethal mutants, and 25 mutants showing the *ina* ERG phenotype and thus presumed to carry new mutant *inaF* alleles. Flies from these 25 lines were crossed to $inaF^{P105p}$ for complementation and confirmed to carry mutant alleles of $inaF^{P105p}$.

This result unequivocally demonstrated that the $inaF^{P105p}$ mutation is caused by a P element insertion.

EXAMPLE 5

Cytogenetic Mapping of the $inaF^{P105p}$ Mutation

To map the *inaF* mutation cytogenetically, a group of deficiency stocks were obtained from the *Drosophila* Stock Center at Indiana University and mated to the *inaF* mutant. The heterozygous F_1 flies that carried Deficiency/*inaF* chromosomes were scored by ERG.

Analysis

A group of deficiency stocks carrying deletions in the 10 C2-E3 region were used to map the $inaF^{P105p}$ mutation cytogenetically (FIG. 7). Three of them did not complement the $inaF^{P105p}$ mutation. Thus, results from cytogenetic mapping independently localized the $inaF^{P105p}$ mutation to the 10 C2-E3 region of the X chromosome, consistent with the P insertion site

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identified by chromosomal *in situ* hybridization as
described in Example 9 below.

5   **EXAMPLE 6**

**An Eye-Specific Clone A23 Fragment
Contains the inaF Gene**

This example shows that, by analyzing genomic
10   Southern and Northern blots, clone A23 was shown to
contain the *inaF* structural gene.

Previous research has shown that the majority of
genes important for phototransduction are expressed
specifically or preferentially in the eyes. Since *inaF*
15   is a vision defective mutant and clone A23 co-localizes
with *inaF*, it is possible that clone A23 may contain
the *inaF* structural gene. However, the 10 C-D-E region
of the X chromosome contains about 500 kb of genomic
DNA which accommodates about 50-100 genes. Therefore
20   it is also possible that clone A23 represents an eye-
specific gene in that region but is unrelated to the
inaF gene. This question was resolved by a combination
of genomic Southern and Northern analyses.

Isolation of Clone A23

25   Several years ago, the Pak laboratory isolated a
pool of *Drosophila* eye-specific clones by subtractive
hybridization. In that method, poly(A)$^+$ RNA extracted
from the heads of wild type flies was reverse
transcribed into cDNA and hybridized with an excess
30   amount of poly(A)$^+$ RNA extracted from the heads of *eyes
absent* (*eya*) mutant flies, all according to standard

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protocols. The eye-specific, single-stranded cDNA

molecules were then separated from the hybridization

mixture by hydroxyapatite chromatography according to

standard protocols and used to screen a genomic library

5   to generate a pool of eye-specific clones. These clones

were further confirmed by dot blots and Northern blots.

The confirmed eye-specific clones were localized on the

polytene chromosomes by chromosomal *in situ*

hybridization. One of them, A23, was localized in the

10   10 D region of the X chromosome.

Genomic Southern Analysis

        Genomic DNA of wild-type flies, the 3B mutators

and the *inaF*^{P105p} flies was isolated by homogenizing

fifteen to twenty flies and using the Puregene kit from

15   Gentra Co. following recommended protocols. 3 μg of

genomic DNA of each type was digested with restriction

enzymes of choice and loaded on a 0.7% agarose gel for

electrophoresis. The agarose gel was denatured in 1.5

M NaCl, 0.5 M NaOH solution for 30 minutes, neutralized

20   in 1 M Tris-Cl, 3 M NaCl, pH 7.5 solution for 40

minutes, blotted overnight onto Hybond-N$^+$ Nylon

membrane (Amersham Co.), and UV cross-linked.

        1 μg of genomic or cDNA fragments was used as

template for ^{32}P-dCTP labeling with random primers.

25   The radioactively labeled probe was purified with a

Sephadex G-50 column.

        Prehybridization treatment was carried out in 0.5

M NaH$_2$PO$_4$, 0.7% SDS, 1% BSA, 0.01 M EDTA solution at

68°C for 3-4 hours, and hybridization was carried out

30   in the same solution at 68°C for 16-20 hours. Washing

was carried out in 0.04 M sodium phosphate buffer, 5%

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SDS, 0.5% BSA, 0.01 M EDTA solution twice for 20
minutes and in 0.04 M sodium phosphate buffer, 1% SDS,
0.01 M EDTA solution twice for 40 minutes. Kodak X-ray
film was used for autoradiography.

5   Northern Analysis

The poly(A)$^+$ RNA was extracted with a PolyATtract-
1000 kit from Promega Co. following their recommended
protocol. 3 μg of poly(A)$^+$ RNA was loaded in each lane
of the agarose gel unless otherwise specified. 1 μg of
10   genomic DNA or cDNA fragment was used as template for
^{32}P-dCTP labeling with random primers.
Prehybridization, hybridization, and washing of
Northern blots were carried out according to the
standard protocol in Sambrook et al., *Molecular Cloning
15   A Laboratory Manual*, 2nd ed. Vol. 1, Cold Spring Harbor
Laboratory Press (1989).

Analysis
        Genomic Southern analyses were used to determine
whether A23 contains DNA fragments flanking the P
20   element insertion that causes the *inaF* mutation. Since
the P insertion is in *inaF*, A23 could not contain the
inaF gene if it were far removed from the P insertion.
        Genomic DNA from wild-type flies, mutator 3B, and
inaF^{P105p} was purified and digested with multiple
25   restriction enzymes, electrophoresed and blotted. A
genomic Southern blot was probed with pCaSpeR3 (FIG.
8). Restriction fragment length polymorphism (RFLP)
was observed and can be interpreted as follows: 1) The
RFLP between wild type and 3B is due to an additional P
30   element in 3B; and 2) The RFLP between 3B and

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$inaF^{P105p}$ is due to the fact that DNA fragments of different sizes flank the P element insertion sites in 3B and $inaF$, and these were detected by the ^{32}P-dCTP labeled pCaSpeR3 probe.

5   Other Genomic Southern blots were probed by ^{32}P-dCTP labeled A23 fragments. Among A23 fragments, fragment 4 (3.6 kb) detected RFLPs between wild-type flies, 3B, and $inaF^{P105p}$ that were similar to those detected by the pCaSpeR3 probe as seen in FIG. 8,

10   except that the EcoRI lanes showed same size signals. This could be due to the fact that the new flanking DNA sequences in $inaF^{P105p}$, though a different species, has the same size as the one flanking the P insertion in 3B. The similarity of the RFLPS between wild-type

15   flies, 3B, and $inaF^{P105p}$ indicates that fragment 4 is likely to contain DNA flanking the P insertion site in $inaF^{P105p}$ (FIG. 9).

     Northern blots were used to examine whether fragment 4 of A23 could detect alterations of

20   transcripts between wild-type flies and $inaF^{P105p}$.

Poly(A)$^+$ RNA was purified from wild-type fly heads, wild-type fly bodies, eya heads, and $inaF^{P105p}$ heads. Because $inaF^{P105p}$ flies undergo age-dependent retinal degeneration, and because confocal microscopy did not

25   detect retinal degeneration in young (<5 days) $inaF^{P105p}$ flies, polyA$^+$ RNA was purified from 1-3 days old $inaF^{P105p}$ flies. Fragment 4 of A23 was used as a probe for Northern analysis, and detected a 3.0 kb eye-

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specific transcript which was drastically reduced in

$inaF^{P105p}$ flies.

Results from the genomic Southern and Northern

analyses jointly indicated that fragment 4 of clone A23

5   contained at least part of the inaF gene and possibly

all of it.

EXAMPLE 7

Isolation of inaF cDNA Clones From a
Drosophila Head cDNA Library

10

Screening Procedure

Fragment 4 of A23, a genomic DNA fragment, was

used as a template for ^{32}P-dCTP labeling with random

15   primers.   The labeled probe was purified with a

Sephadex G-50 column and was used to screen 5 X 10^5

plaque forming units (pfu) of a *Drosophila* head cDNA

Library, a gift from Dr. Erich Buchner at Wurzburg

University in Germany. The cDNA library screening was

20   carried out according to a standard protocol in

Sambrook et al., *Molecular Cloning A Laboratory Manual*,

2nd ed. Vol. 1, Cold Spring Harbor Laboratory Press

(1989).

25   Analysis

10 positively hybridizing cDNA clones were

obtained and purified as single plaques.   Cross

hybridization among these clones demonstrated that they

all belong to the same class of cDNA.   cDNA#1 had the

30   biggest insert and thus was used for further

experiments.   The insert of cDNA#1 was labeled with

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biotin-dUTP and used as a probe for chromosomal *in situ* hybridization and detected a hybridization signal in the 10 C2-E3 region as described in Example 8. The insert was also used to probe a genomic Southern blot and detected the same RFLP as those revealed by pCaSpeR3 and A23 probes. Finally, the insert was labeled with ^{32}P-dCTP and used to probe a Northern blot.

Three µg of polyA+ RNA of each sample was loaded on the gel. cDNA#1 insert was labeled with ^{32}P-dCTP. A 3.0 kb transcript was detected in the poly(A)$^+$ RNA from wild-type fly heads but not that from wild-type fly bodies and *eya* heads, indicating that the 3.0 kb transcript is eye specific (FIG. 11). The same transcript was absent from the poly(A)$^+$ RNA from inaF^{P105P}, indicating that the cDNA most likely contains the inaF gene. The same blot was boiled to eliminate the radioactive probe and used again for a control experiment in which RP49, a ribosomal protein universally expressed in all tissues, was used as a probe. These lines of evidence all suggested that cDNA#1 corresponds to the *inaF* gene.

EXAMPLE 8

Chromosomal Location of the *inaF*^{P105p} P Insertion

To localize the *inaF*^{P105p} P insertion site on polytene chromosomes, pCaSpeR3, the P element employed in the local hopping mutagenesis described in Example 1, was used as a template for synthesizing biotin-dUTP probes for chromosomal *in situ* hybridization to the

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polytene chromosomes of *inaF*^{P105p}. In this case,
however, a genomic DNA fragment from clone A23,
discussed in Example 6, and a cDNA fragment of cDNA#1
were used as templates for the biotin-dUTP labelling.

5   The probe hybridized to 10 C2-E3 (FIG. 10). The signal
at 10 C2-E3 was due to detection of the new P
insertion.

EXAMPLE 9
Sequence of *inaF* cDNA

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Sequencing cDNA clone #1
        cDNA clone #1 was partially digested with EcoRI

and subcloned into the pBluescript-SK^{+} vector. T3 and
T7 primers were used for initial sequencing reactions,

15   and internal sequencing primers were designed and
synthesized according to the sequence data obtained
from each gel reading. The sequencing reactions were
carried out at the DNA Sequencing Center at Iowa State
University, Ames, Iowa. Both strands were sequenced,

20   and every nucleotide has been confirmed from at least
three independent reactions. The sequence of cDNA
clone 1 is set forth in SEQ ID:1. A similar nucleotide
sequence, differing only in certain 5′ regions, and
including a linker sequence at the 3′ end, is set forth

25   in SEQ ID:2.

Analysis

        The cDNA has a poly(A)$^{+}$ tail immediately before
the 3′-end EcoRI cloning site, and a consensus

polyadenylation signal (AATAAA) preceding the polyA$^+$. This indicates that the 3' end of the cDNA is intact.

At the 5' end, the translation start site was determined on the basis that an in-frame stop codon is present about 15 amino acids upstream to the methionine assigned as the +1 site.

The putative protein has 241 amino acids with an estimated molecular weight of 26 kd. It appears to be a soluble protein since the Kyte-Doolittle plot did not reveal any hydrophobic segments which can serve as transmembrane domains. A BLAST search of the NCBI, EMBL and SWISSPORT databases did not find significant homology with any known proteins. A MOTIF search identified two potential glycosylation sites (position 18 and 103) and a potential PKC phosphorylation site (position 144).

FIG. 12 shows a restriction map of inaF cDNA and of the corresponding genomic region in the A23 clone and three inaF mutants.

EXAMPLE 10

Immunodetection of the TRP protein

To determine if inaF mutations affect the amount of the TRP protein, Western blot analyses were performed. The blot was probed with a monoclonal anti-TRP antibody described in Pollock, *J. Neurosci.* 15:3747-3760 (1995). Results showed that the TRP protein is reduced to about 15% and 10% of the wild type level in *inaF^{P105P}* and *inaF^{P106x}*, respectively, at 1 day post-eclosion (FIG. 13). The reductions are not due to non-specific reductions of retinal proteins. Other retinal proteins examined [rhodopsin, PLCB

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(NORPA), and InaD) did not show similar reductions at
this age (data not shown), nor were there any signs of
retinal degradation in such young flies.

EXAMPLE 11

Recombinant Expression Vectors Encoding InaF

A glutathione-S-transferase-InaF polypeptide (GST-
InaF) fusion construct was made by ligating the *inaF*
coding region in frame with the glutathione transferase
gene in the pGEX-KG vector [(Guan and Dixon, *Anal.
Biochem.*, 192:262-267 (1991)]. Following
transformation of bacteria (E. coli BL-21), over
expression of the fusion protein was achieved by
induction with IPTG. The fusion protein was partially
purified by using immobilized glutathione [Guan and
Dixon (1991), cited above]. Further purification can
be achieved by ion exchange chromatography. In order
to obtain purified InaF protein, the fusion protein can
be digested with thrombin (Sigma) and the InaF protein
can be eluted from an immobilized glutathione agarose
column as known in the art.

Biological Deposit Under The Budapest Treaty

A deposit of inaF cDNA, designated as inaF cDNA-
1/XL-1 Blue was deposited with the American Type
Culture Collection, 10801 University Blvd., Manassas,
VA 20110-2209. The deposit is Epicurian Coli XL-1 Blue
(Stratagene) harboring inaF cDNA (SEQ ID:1, nucleotides
314 to 1036) from Drosophila melanogaster (Berlin) in a

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pBluescript II KS (Stratagene) vector. The accession
number is ATCC 207232.

        While the invention has been illustrated and
5   described in detail in the drawings and foregoing
    description, the same is to be considered as
    illustrative and not restrictive in character, it being
    understood that only the preferred embodiment has been
    shown and described and that all changes and
10 modifications that come within the spirit of the
    invention are desired to be protected. In addition,
    all references cited herein are indicative of the level
    of skill in the art and are hereby incorporated by
    reference in their entirety.
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CLAIMS

What is claimed is:

5   1.   An isolated nucleic acid molecule, comprising
a nucleotide sequence encoding a protein functioning in
regulating calcium ion entry into cells, said
nucleotide sequence having substantial similarity to
the nucleotide sequence set forth in SEQ ID:1 from
10   nucleotide 314 to nucleotide 1036.

        2.   The molecule of claim 1, wherein said
nucleotide sequence is comprised of the nucleotide
sequence set forth in SEQ ID:1 from nucleotide 314 to
15   nucleotide 1036.

        3.   The molecule of claim 1, wherein said protein
is comprised of an amino acid sequence having at least
about 30% identity with the amino acid sequence set
20   forth in SEQ ID:1.

        4.   The molecule of claim 1, wherein said protein
is comprised of an amino acid sequence having at least
about 50% identity with the amino acid sequence set
25   forth in SEQ ID:1.

        5.   The molecule of claim 1, wherein said protein
is comprised of an amino acid sequence set forth in SEQ
ID:1.

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6.   The molecule of claim 1, wherein said protein is comprised of an amino acid sequence set forth in SEQ ID:1 or a sufficiently similar sequence thereto to exhibit the ability to regulate calcium ion entry into
5   cells.

7.   An isolated nucleic acid molecule, comprising a nucleotide sequence encoding a protein functioning in regulating calcium ion entry into cells, said
10   nucleotide sequence having the sequence set forth in SEQ ID:1 from nucleotide 314 to nucleotide 1036.

8.   An isolated nucleic acid molecule, comprising a nucleotide sequence having at least about 80%
15   identity to a 400 nucleotide long sequence within the sequence set forth in SEQ ID:1 from nucleotide 301 to nucleotide 1036, said nucleotide sequence from nucleotide 301 to nucleotide 1036 encoding a protein functioning in regulating calcium entry into cells.
20

9.   A recombinant nucleic acid molecule, comprising a nucleotide sequence encoding a protein functioning in regulating calcium ion entry into cells, said nucleotide sequence having substantial similarity
25   to the sequence set forth in SEQ ID:1 from nucleotide 314 to nucleotide 1036.

10.   The molecule of claim 9, wherein said nucleotide sequence is comprised of the sequence set
30   forth in SEQ ID:1 from nucleotide 314 to nucleotide 1036.

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11.   The molecule of claim 9, wherein said protein is comprised of an amino acid sequence having at least about 30% identity with the amino acid sequence set forth in SEQ ID:1.

12.   The molecule of claim 9, wherein said protein is comprised of an amino acid sequence having at least about 50% identity with the amino acid sequence set forth in SEQ ID:1.

13.   The molecule of claim 9, wherein said protein is comprised of an amino acid sequence set forth in SEQ ID:1.

14.   The molecule of claim 9, wherein said protein is comprised of an amino acid sequence set forth in SEQ ID:1 or a sufficiently similar sequence thereto to exhibit the ability to regulate calcium ion entry into cells.

15.   The molecule of claim 9, further comprising a promoter operably linked to a terminal 5' end of said nucleotide sequence.

16.   The molecule of claim 15, wherein said promoter is selected from the group consisting of a constitutive promoter, an inducible promoter, and a cell-specific promoter.

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17.   A recombinant nucleic acid molecule,
comprising a nucleotide sequence encoding a protein
functioning in regulating calcium ion entry into cells,
said nucleotide sequence having the sequence set forth
5   in SEQ ID:1 from nucleotide 314 to nucleotide 1036.

18.   A host cell, comprising an introduced nucleic
acid molecule having a nucleotide sequence of
substantial similarity to the nucleotide sequence set
10   forth in SEQ ID:1 from nucleotide 314 to nucleotide
1036, said nucleotide sequence encoding a protein
functioning in regulating calcium ion entry into cells.

19.   The host cell of claim 18, wherein said
15   nucleotide sequence is comprised of the nucleotide
sequence set forth in SEQ ID:1 from nucleotide 314 to
nucleotide 1036.

20.   The host cell of claim 18, wherein said
20   protein is comprised of an amino acid sequence having
at least about 30% identity with the amino acid
sequence set forth in SEQ ID:1.

21.   The host cell of claim 18, wherein said
25   protein is comprised of an amino acid sequence having
at least about 50% identity with the amino acid
sequence set forth in SEQ ID:1.

22.   The host cell of claim 18, wherein said
30   protein is comprised of an amino acid sequence set
forth in SEQ ID:1.

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23. The host cell of claim 18, wherein said protein is comprised of an amino acid sequence set forth in SEQ ID:1 or a sufficiently similar sequence thereto to exhibit the ability to regulate calcium ion
5   entry into cells.

24. A host cell, comprising an introduced nucleic acid molecule having a nucleotide sequence set forth in SEQ ID:1 from nucleotide 314 to nucleotide
10 1036, said nucleotide sequence encoding a protein functioning in regulating calcium ion entry into cells.

25. A purified InaF protein.

15 26. A purified protein, said protein having an amino acid sequence having at least about 30% identity to the amino acid sequence set forth in SEQ ID:1, said protein functioning in regulating calcium ion entry into cells.
20

27. The protein of claim 26, wherein said protein has an amino acid sequence as set forth in SEQ ID:1.

28. The protein of claim 26, wherein said protein
25 has an amino acid sequence having at least about 50% identity to the amino acid sequence set forth in SEQ ID:1.

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29. The protein of claim 26, wherein said protein
has an amino acid sequence as set forth in SEQ ID:1 or
a sufficiently similar amino acid sequence thereto to
exhibit the ability to regulate calcium ion entry into
5   cells.

30. A purified protein, said protein having an
amino acid sequence set forth in SEQ ID:1, said protein
functioning in regulating calcium ion influx into
10   cells.

31. A purified protein, said protein having an
amino acid sequence encoded by a nucleic acid molecule
having a nucleotide sequence of substantial similarity
15   to the nucleotide sequence set forth in SEQ ID:1 from
nucleotide 314 to nucleotide 1036, said protein
functioning in regulating calcium ion entry into cells.

32. A recombinant protein, comprising:
20   an amino acid sequence having at least about 30%
identity to the amino acid sequence set forth in SEQ
ID:1, said protein functioning in regulating calcium
ion entry into cells.

25   33. The protein of claim 32, wherein said protein
has an amino acid sequence having at least about 50%
identity to the amino acid sequence set forth in SEQ
ID:1.

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34.   A method of expressing an InaF protein, said method comprising:

(a)   introducing into a host cell a nucleotide sequence encoding a protein functioning in regulating calcium ion entry into cells, said nucleotide sequence having substantial similarity to the sequence set forth in SEQ ID:1 from nucleotide 314 to nucleotide 1036; and

(b)   culturing under conditions to achieve expression of said protein.

35.   The method of claim 34, wherein said nucleotide sequence is comprised of the nucleotide sequence set forth in SEQ ID:1 from nucleotide 314 to nucleotide 1036.

36.   The method of claim 34, wherein said protein is comprised of an amino acid sequence having at least about 30% identity with the amino acid sequence set forth in SEQ ID:1.

37.   The method of claim 34, wherein said protein is comprised of an amino acid sequence having at least about 50% identity with the amino acid sequence set forth in SEQ ID:1.

38.   The method of claim 34, wherein said protein is comprised of an amino acid sequence set forth in SEQ ID:1.

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39.   The method of claim 34, wherein said protein
is comprised of an amino acid sequence set forth in SEQ
ID:1 or a sufficiently similar sequence thereto to
exhibit the ability to regulate calcium entry into
5   cells.

40.   The method of claim 34, wherein said
nucleotide sequence is inserted in a vector.

10   41.   The method of claim 40, wherein said vector
is a plasmid vector.

42.   A method of expressing an InaF protein, said
method comprising:
15   (a)   introducing into a host cell a
recombinant nucleic acid molecule comprising a
nucleotide sequence encoding a protein functioning in
regulating calcium ion entry into cells, said
nucleotide sequence having substantial similarity to
20   the sequence set forth in SEQ ID:1 from nucleotide 314
to nucleotide 1036; and
(b)   culturing under conditions to achieve
expression of said protein.

25   43.   The method of claim 42, wherein said
nucleotide sequence is comprised of the nucleotide
sequence set forth in SEQ ID:1 from nucleotide 314 to
nucleotide 1036.

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 99 Arg Pro Ser His His Ala His Gly His Val His Gly Glu Arg Arg Asn
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103 Gly Ala Gln Ser Gly Gly Arg Arg Arg Thr Thr Thr Ala Ser Val Ser
104                 210                 215                 220
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107 Gly Tyr Glu Ala Gln Thr Tyr Leu Asn Pro Phe Leu Thr Gly Glu Leu
108                 225                 230                 235
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111 Ile Phe Glu Lys
112             240
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144 aaacagtaac cagtaactag taaccaatta ccaattacca gtaacccatc caaggagtat     2036
146 acccccttg caaacgggga agcggataaa tgtcactaga attcagcatc atcagattga     2096
148 atcacacaca atcctagtcg cctcacgcga agagaactat gtcatgatca gatatcggtg     2156
150 tatgcattct atattatgta cttcgaaata tgtaatttat taagtttcg ctatacttt      2216
152 cattcaaatt ggcaaaaacc aattcaaagg ttttcaatat tttcgaaaag cattttaggc     2276
154 tttctatgta acgtatgttt ttcaaacaaa atattagttt ttgaaacttt attatcggat     2336
156 aaacaaatgt aagccaaatt acaacgttta tgatactcca aagattcgca ctataaagtg     2396
158 gcctaaaaat agctgacgca ttagccatag gcgcttcgct tctcaagata aaacctgggc     2456
160 gtgctcaact caagaacaaa tatgtggtta tatacatata tacatatatg gggcatataa     2516
162 ccgatgtgtg acgtgacatt ggctcgttct attcacatac ttaaacacta aatgcaaacc     2576
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164 tatcaaaaac caactacact aagcgaaaag cggcagagat agttaaggaa agtggtcaag    2636
166 agaggacgag agagagagag agagaaagtg aaagtgaaag ggagagatag taaaactgca    2696
168 tctgcatcca aagacacgag aattgaattc atcaataata acatacgtat aaacgatatg    2756
170 catacgatat agaattgaat ctgtaactga tgggcatata ccgcatatat atcttatata    2816
172 ccgcatatat cttatatatg tataccaaga aaaacaaagt catttggcaa taataaagca    2876
174 tagcaaacaa caataaaaaa aaggaattc                                      2905
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181 <211> LENGTH: 3112
183 <212> TYPE: DNA
185 <213> ORGANISM: Drosophila melanogaster
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195 gtcggcactg atggccaatc tggccgatgt ggtcaaggag gccaaggatg aggagatccc     180
197 gatgcccaaa tcgaatgact tcttcgagtc caagaccttc cgcttgctca ccctgatgct     240
199 ctacatgggc ggcgttagcg gcatgggctt gactctggct gtctactacc tgttcatctg     300
201 ggattcacgc atgccgccgc tgcccgtgtt caagcacacg catccgattg gctaggatcg     360
203 gatggctatc atttagttag ccatccatct cccagcgaca tcagtgaccg gaattggata     420
205 aaattgtgat acccgagaaa atagccattc gcctcctgaa ctcatcggaa gtgaccgcgg     480
207 agcagttcta caagcacatc ctcgagcagt accgcatcct cagccac                   527
```

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210 atg caa cag cag cgc cag caa ctg ctg cag cgc caa cat ctc caa ctg       575
211 Met Gln Gln Gln Arg Gln Gln Leu Leu Gln Arg Gln His Leu Gln Leu
212 1               5                   10                  15

215 cag cag ctg gag gca aac aat cgc ttc cag gag gtc ttt gcc acg gcc       623
216 Gln Gln Leu Glu Ala Asn Asn Arg Phe Gln Glu Val Phe Ala Thr Ala
217             20                  25                  30

219 acc atc att cag gca cat ccg cat ccc cat cca cat ccc agg gag ccg       671
220 Thr Ile Ile Gln Ala His Pro His Pro His Pro His Pro Arg Glu Pro
221         35                  40                  45

223 ccc aag aag ccg ctt tta gga cca tat agc ccg caa ccc ggc aac ata       719
224 Pro Lys Lys Pro Leu Leu Gly Pro Tyr Ser Pro Gln Pro Gly Asn Ile
225     50                  55                  60

227 agt cac gct atg ggt ggt gat cag ttg gat gca gaa acg gaa cag ggt       767
228 Ser His Ala Met Gly Gly Asp Gln Leu Asp Ala Glu Thr Glu Gln Gly
229 65                  70                  75                  80

231 cac atg cct cta atc ctg gat acc tca ccg ccg gtc gaa gta acc gga       815
232 His Met Pro Leu Ile Leu Asp Thr Ser Pro Pro Val Glu Val Thr Gly
233                 85                  90                  95

235 atg ggt cac ctg aag cgg aag aca cat cgc ggt cac tac aaa cat cat       863
236 Met Gly His Leu Lys Arg Lys Thr His Arg Gly His Tyr Lys His His
237             100                 105                 110

239 aga gcc cga gcc ggt ggt caa aag aaa ctg tcc att gcc aat tcg atg       911
240 Arg Ala Arg Ala Gly Gly Gln Lys Lys Leu Ser Ile Ala Asn Ser Met
241         115                 120                 125

243 gcc agc tcc acg ccg agc acc aca gcc gga gga gat gcg tca atg gcc       959
244 Ala Ser Ser Thr Pro Ser Thr Thr Ala Gly Gly Asp Ala Ser Met Ala
245     130                 135                 140

247 act gcg gcc act ttg cca cat ggt tat atg gac gct cca cta aat ccg      1007
248 Thr Ala Ala Thr Leu Pro His Gly Tyr Met Asp Ala Pro Leu Asn Pro
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RAW SEQUENCE LISTING   DATE: 08/07/2001
PATENT APPLICATION: US/09/700,869   TIME: 17:38:39

Input Set : A:\Pto.amc
Output Set: N:\CRF3\08072001\I700869.raw

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252 Ala Ala Gly Thr Ile Val Gln Ala Pro Gln Leu Gln Leu Tyr Thr Ser
253                  165               170               175
255 atg ccc att cca ctg atc ctg agt ccc agc gac gaa aag cgt cct tcg    1103
256 Met Pro Ile Pro Leu Ile Leu Ser Pro Ser Asp Glu Lys Arg Pro Ser
257                  180               185               190
259 cac cac gcc cac gga cat gtc cat ggc gag agg cgg aac ggg gcg caa    1151
260 His His Ala His Gly His Val His Gly Glu Arg Arg Asn Gly Ala Gln
261              195               200               205
263 tcc ggc ggc cgg cga agg acc acg acg gca tcg gtt tct ggc tac gag    1199
264 Ser Gly Gly Arg Arg Arg Thr Thr Thr Ala Ser Val Ser Gly Tyr Glu
265          210               215               220
269 gcg cag acc tac ctc aat ccg ttt ctc acc ggc gag ctg atc ttc gag    1247
270 Ala Gln Thr Tyr Leu Asn Pro Phe Leu Thr Gly Glu Leu Ile Phe Glu
271 225                  230               235               240
273 aag taagggactg cacccagatc aggaaacgtc gcggttcatt gttttttttt         1300
274 Lys
276 tttttttttt tttaatgcat gtagagggat atacactaca gtcaagatcg gaattggaga   1360
278 ttagctcata gaaatggtaa ctgcccaaga aacaaaaaaa gaaatgacta acaaatgggc   1420
280 aataataccc tcaatacctt gtcataccta tttgaatgga gaaataactc agttaagctc   1480
282 agtactggca taagcatggg gaaaatattt caattaatca gttcgagtag atatgttttc   1540
284 caaattgata gcgatattag acatttcata ttgaaattta caggtacaaa tataatctca   1600
286 gttaatgcgt agaatgcgtt tgcaattgaa caaatttaac gttttatagc aagaacttag   1660
288 gaacaaaagt tgtaatcgct tattctataa taatgttaac taaagccaaa gcaagtagat   1720
290 cggttgtata actcatttct gctatagata agtcttgact tgtatcagta ctgagctata   1780
292 aattggtcca tatatacgta gccaggcctg gccacatata gaggttaata aacgttatgt   1840
294 actgcaaaag gatagttgaa accatagcta aacgatagtc gatgtaccaa ccacactcca   1900
296 ctccaatcca atccaatcca atccactcaa atcaattcaa aacaacacac tcgtaaggga   1960
298 cacacactca cgcataatac gggacccact tcagtagaaa gtcacgat atcagcgatc   2020
300 acggatcacg aattacggat cacggaatac gtatcacgga tcacacaggc ggctcacctc   2080
302 accaagctca gcagcaaact caccccacct aggacactgc ttccaggcag ctagcgaacg   2140
304 ctacaccaac tacaataatt agccaaccct agagtaatca gtttaccagt aaacagtaac   2200
306 cagtaactag taaccaatta ccaattacca gtaacccatc caaggagtat accccccttg   2260
308 caaacgggga agcggataaa tgtcactaga attcagcatc atcagattga atcacacaca   2320
310 atcctagtcg cctcacgcga agagaactat gtcatgatca gatatcggtg tatgcattct   2380
312 atattatgta cttcgaaata tgtaatttat taagttttcg ctatacttt cattcaaatt   2440
314 ggcaaaaacc aattcaaagg ttttcaatat tttcgaaaag cattttaggc tttctatgta   2500
316 acgtatgttt ttcaaacaaa atattagttt ttgaacttt attatcggat aaacaaatgt   2560
318 aagccaaatt acaacgttta tgatactcca aagattcgca ctataaagtg gcctaaaaat   2620
320 agctgacgca ttagccatag gcgcttcgct tctcaagata aaacctgggc gtgctcaact   2680
322 caagaacaaa tatgtggtta tatacatata tacatatatg gggcatataa ccgatgtgtg   2740
324 acgtgacatt ggctcgttct attcacatac ttaaacacta aatgcaaacc tatcaaaaac   2800
326 caactacact aagcgaaaag cggcagagat agttaaggaa agtggtcaag agaggacgag   2860
328 agagagagag agagaaagtg aaagtgaaag ggagagatag taaaactgca tctgcatcca   2920
330 aagacacgag aattgaattc atcaataata acatacgtat aaacgatatg catacgatat   2980
332 agaattgaat ctgtaactga tgggcatata ccgcatatat atcttatata ccgcatatat   3040
334 cttatatatg tataccaaga aaacaaagt catttggcaa taataaagca tagcaaacaa   3100
336 caataaaaaa aa                                                       3112
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VERIFICATION SUMMARY   DATE: 08/07/2001
PATENT APPLICATION: **US/09/700,869**   TIME: 17:38:40

Input Set : **A:\Pto.amc**
Output Set: **N:\CRF3\08072001\I700869.raw**

L:11 M:270 C: Current Application Number differs, Replaced Current Application Number
L:13 M:271 C: Current Filing Date differs, Replaced Current Filing Date

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           RAW SEQUENCE LISTING            DATE: 07/15/2001
           PATENT APPLICATION:  US/09/700,869     TIME: 20:15:54

           Input Set : A:\PUR-92SEQ.doc
           Output Set: N:\CRF3\07152001\I700869.raw
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W--> 1 (PUR-092-PCT:46662/7024-384 v.7) *delete*   Does Not Comply
                                                     Corrected Diskette Needed
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      5 <110> APPLICANT: Pak, William L.
      6        Li, Chenjian
      7        Geng, Chaoxian
      9 <120> TITLE OF INVENTION: Calcium Channel Regulators
     11 <130> FILE REFERENCE: 7024-384/PUR-92
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C--> 15 **<141> CURRENT FILING DATE: 2001-05-24**
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     21 <150> PRIOR APPLICATION NUMBER: U.S. 60/098,072
     23 <151> PRIOR FILING DATE: 1998-08-27
     25 <160> NUMBER OF SEQ ID NOS: 2
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     29 <210> SEQ ID NO: 1
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     37 <400> SEQUENCE: 1
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     44 cacacgcatc cgattggcta ggatcggatg gctatcattt agttagccat ccatctccca   180
     46 gcgacatcag tgaccggaat tggataaaat tgtgataccc gagaaaatag ccattcgcct   240
     48 cctgaactca tcggaagtga ccgcggagca gttctacaag cacatcctcg agcagtaccg   300
     50 catcctcagc cac atg caa cag cag cgc cag caa ctg ctg cag cgc caa cat   352
     51               Met Gln Gln Gln Arg Gln Gln Leu Leu Gln Arg Gln His
     52                1               5                  10
     54 ctc caa ctg cag cag ctg gag gca aac aat cgc ttc cag gag gtc ttt      400
     55 Leu Gln Leu Gln Gln Leu Glu Ala Asn Asn Arg Phe Gln Glu Val Phe
     56     15              20                  25
     60 gcc acg gcc acc atc att cag gca cat ccg cat ccc cat cca cat ccc      448
     61 Ala Thr Ala Thr Ile Ile Gln Ala His Pro His Pro His Pro His Pro
     62 30                  35                  40                  45
     64 agg gag ccg ccc aag aag ccg ctt tta gga cca tat agc ccg caa ccc      496
     65 Arg Glu Pro Pro Lys Lys Pro Leu Leu Gly Pro Tyr Ser Pro Gln Pro
     66                 50                  55                  60
     68 ggc aac ata agt cac gct atg ggt ggt gat cag ttg gat gca gaa acg      544
     69 Gly Asn Ile Ser His Ala Met Gly Gly Asp Gln Leu Asp Ala Glu Thr
     70                 65                  70                  75
     72 gaa cag ggt cac atg cct cta atc ctg gat acc tca ccg ccg gtc gaa      592
     73 Glu Gln Gly His Met Pro Leu Ile Leu Asp Thr Ser Pro Pro Val Glu
     74             80                  85                  90
     76 gta acc gga atg ggt cac ctg aag cgg aag aca cat cgc ggt cac tac      640
     77 Val Thr Gly Met Gly His Leu Lys Arg Lys Thr His Arg Gly His Tyr
     78        95                  100                 105
     80 aaa cat cat aga gcc cga gcc ggt ggt caa aag aaa ctg tcc att gcc      688
     81 Lys His His Arg Ala Arg Ala Gly Gly Gln Lys Lys Leu Ser Ile Ala
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 84 aat tcg atg gcc agc tcc acg ccg agc acc aca gcc gga gga gat gcg        736
 85 Asn Ser Met Ala Ser Ser Thr Pro Ser Thr Thr Ala Gly Gly Asp Ala
 86             130                 135                 140
 88 tca atg gcc act gcg gcc act ttg cca cat ggt tat atg gac gct cca        784
 89 Ser Met Ala Thr Ala Ala Thr Leu Pro His Gly Tyr Met Asp Ala Pro
 90             145                 150                 155
 92 cta aat ccg gcg gca gga acc att gtc cag gca cca caa ctg cag cta        832
 93 Leu Asn Pro Ala Ala Gly Thr Ile Val Gln Ala Pro Gln Leu Gln Leu
 94         160                 165                 170
 96 tac acc tcg atg ccc att cca ctg atc ctg agt ccc agc gac gaa aag        880
 97 Tyr Thr Ser Met Pro Ile Pro Leu Ile Leu Ser Pro Ser Asp Glu Lys
 98     175                 180                 185
100 cgt cct tcg cac cac gcc cac gga cat gtc cat ggc gag agg cgg aac        928
101 Arg Pro Ser His His Ala His Gly His Val His Gly Glu Arg Arg Asn
102 190                 195                 200                 205
104 ggg gcg caa tcc ggc ggc cgg cga agg acc acg acg gca tcg gtt tct        976
105 Gly Ala Gln Ser Gly Gly Arg Arg Arg Thr Thr Thr Ala Ser Val Ser
106                 210                 215                 220
108 ggc tac gag gcg cag acc tac ctc aat ccg ttt ctc acc ggc gag ctg       1024
109 Gly Tyr Glu Ala Gln Thr Tyr Leu Asn Pro Phe Leu Thr Gly Glu Leu
110             225                 230                 235
112 atc ttc gag aag taagggactg cacccagatc aggaaacgtc gcggttcatt           1076
113 Ile Phe Glu Lys
114         240
116 gttttttttt tttttttttt tttaatgcat gtagagggat atacactaca gtcaagatcg     1136
118 gaattggaga ttagctcata gaaatggtaa ctgcccaaga aacaaaaaaa gaaatgacta     1196
120 acaaatgggc aataataccc tcaatacctt gtcataccta tttgaatgga gaaataactc     1256
122 agttaagctc agtactggca taagcatggg gaaaatattt caattaatca gttcgagtag     1316
124 atatgttttc caaattgata gcgatattag acatttcata ttgaaattta caggtacaaa     1376
126 tataatctca gttaatgcgt agaatgcgtt tgcaattgaa caaatttaac gttttatagc     1436
128 aagaacttag gaacaaaagt tgtaatcgct tattctataa taatgttaac taaagccaaa     1496
130 gcaagtagat cggttgtata actcatttct gctatagata agtcttgact tgtatcagta     1556
132 ctgagctata aattggtcca tatatacgta gccaggcctg gccacatata gaggttaata     1616
134 aacgttatgt actgcaaaag gatagttgaa accatagcta aacgatagtc gatgtaccaa     1676
136 ccacactcca ctccaatcca atccaatcca atccactcaa atcaattcaa aacaacacac     1736
138 tcgtaaggga cacacactca cgcataatac gggacccact tcagtagaaa gtcacacgat     1796
140 atcagcgatc acggatcacg aattacggat cacggaatac gtatcacgga tcacacaggc     1856
142 ggctcacctc accaagctca gcagcaaact caccccacct aggacactgc ttccaggcag     1916
144 ctagcgaacg ctacaccaac tacaataatt agccaaccct agagtaatca gtttaccagt     1976
146 aaacagtaac cagtaactag taaccaatta ccaattacca gtaacccatc caaggagtat     2036
148 accccccttg caaacgggga agcggataaa tgtcactaga attcagcatc atcagattga     2096
150 atcacacaca atcctagtcg cctcacgcga agagaactat gtcatgatca gatatcggtg     2156
152 tatgcattct atattatgta cttcgaaata tgtaatttat taagttttcg ctatactttt     2216
154 cattcaaatt ggcaaaaacc aattcaaagg ttttcaatat tttcgaaaag cattttaggc     2276
156 tttctatgta acgtatgttt ttcaaacaaa atattagttt ttgaaacttt attatcggat     2336
158 aaacaaatgt aagccaaatt acaacgttta tgatactcca aagattcgca ctataaagtg     2396
160 gcctaaaaat agctgacgca ttagccatag gcgcttcgct tctcaagata aaacctgggc     2456
162 gtgctcaact caagaacaaa tatgtggtta tatacatata tacatatatg gggcatataa     2516
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RAW SEQUENCE LISTING   DATE: 07/15/2001
PATENT APPLICATION: US/09/700,869   TIME: 20:15:54

Input Set : A:\PUR-92SEQ.doc
Output Set: N:\CRF3\07152001\I700869.raw

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166 tatcaaaaac caactacact aagcgaaaag cggcagagat agttaaggaa agtggtcaag     2636
168 agaggacgag agagagagag agagaaagtg aaagtgaaag ggagagatag taaaactgca     2696
170 tctgcatcca aagacacgag aattgaattc atcaataata acatacgtat aaacgatatg     2756
172 catacgatat agaattgaat ctgtaactga tgggcatata ccgcatatat atcttatata     2816
174 ccgcatatat cttatatatg tataccaaga aaaacaaagt catttggcaa taataaagca     2876
176 tagcaaacaa caataaaaaa aaggaattc                                       2905
181 <210> SEQ ID NO: 2
183 <211> LENGTH: 3112
185 <212> TYPE: DNA
187 <213> ORGANISM: Drosophila melanogaster
189 <400> SEQUENCE: 2
193 gaattgtgtt cagttcgttc gaagaggcgg ttacggttgc gattggccac ctttttccat       60
195 atcgcttggg tcattcagca cattctcgaa ataaataaga agcggcataa tgagcggacc      120
197 gtcggcactg atggccaatc tggccgatgt ggtcaaggag gccaaggatg aggagatccc      180
199 gatgcccaaa tcgaatgact tcttcgagtc caagaccttc cgcttgctca ccctgatgct      240
201 ctacatgggc ggcgttagcg gcatgggctt gactctggct gtctactacc tgttcatctg      300
203 ggattcacgc atgccgccgc tgcccgtgtt caagcacacg catccgattg gctaggatcg      360
205 gatggctatc atttagttag ccatccatct cccagcgaca tcagtgaccg gaattggata      420
207 aaattgtgat acccgagaaa atagccattc gcctcctgaa ctcatcggaa gtgaccgcgg      480
209 agcagttcta caagcacatc ctcgagcagt accgcatcct cagccac                   527
```

```
212 atg caa cag cag cgc cag caa ctg ctg cag cgc caa cat ctc caa ctg       575
213 Met Gln Gln Gln Arg Gln Gln Leu Leu Gln Arg Gln His Leu Gln Leu
214 1               5                   10                  15

217 cag cag ctg gag gca aac aat cgc ttc cag gag gtc ttt gcc acg gcc       623
218 Gln Gln Leu Glu Ala Asn Asn Arg Phe Gln Glu Val Phe Ala Thr Ala
219             20                  25                  30

221 acc atc att cag gca cat ccg cat ccc cat cca cat ccc agg gag ccg       671
222 Thr Ile Ile Gln Ala His Pro His Pro His Pro His Pro Arg Glu Pro
223             35                  40                  45

225 ccc aag aag ccg ctt tta gga cca tat agc ccg caa ccc ggc aac ata       719
226 Pro Lys Lys Pro Leu Leu Gly Pro Tyr Ser Pro Gln Pro Gly Asn Ile
227         50                  55                  60

229 agt cac gct atg ggt ggt gat cag ttg gat gca gaa acg gaa cag ggt       767
230 Ser His Ala Met Gly Gly Asp Gln Leu Asp Ala Glu Thr Glu Gln Gly
231 65                  70                  75                  80

233 cac atg cct cta atc ctg gat acc tca ccg ccg gtc gaa gta acc gga       815
234 His Met Pro Leu Ile Leu Asp Thr Ser Pro Pro Val Glu Val Thr Gly
235             85                  90                  95

237 atg ggt cac ctg aag cgg aag aca cat cgc ggt cac tac aaa cat cat       863
238 Met Gly His Leu Lys Arg Lys Thr His Arg Gly His Tyr Lys His His
239             100                 105                 110

241 aga gcc cga gcc ggt ggt caa aag aaa ctg tcc att gcc aat tcg atg       911
242 Arg Ala Arg Ala Gly Gly Gln Lys Lys Leu Ser Ile Ala Asn Ser Met
243         115                 120                 125

245 gcc agc tcc acg ccg agc acc aca gcc gga gga gat gcg tca atg gcc       959
246 Ala Ser Ser Thr Pro Ser Thr Thr Ala Gly Gly Asp Ala Ser Met Ala
247         130                 135                 140

249 act gcg gcc act ttg cca cat ggt tat atg gac gct cca cta aat ccg      1007
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RAW SEQUENCE LISTING   DATE: 07/15/2001
PATENT APPLICATION: US/09/700,869   TIME: 20:15:54

Input Set : A:\PUR-92SEQ.doc
Output Set: N:\CRF3\07152001\I700869.raw

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254 Ala Ala Gly Thr Ile Val Gln Ala Pro Gln Leu Gln Leu Tyr Thr Ser
255                 165                 170                 175
257 atg ccc att cca ctg atc ctg agt ccc agc gac gaa aag cgt cct tcg      1103
258 Met Pro Ile Pro Leu Ile Leu Ser Pro Ser Asp Glu Lys Arg Pro Ser
259                 180                 185                 190
261 cac cac gcc cac gga cat gtc cat ggc gag agg cgg aac ggg gcg caa      1151
262 His His Ala His Gly His Val His Gly Glu Arg Arg Asn Gly Ala Gln
263         195                 200                 205
265 tcc ggc ggc cgg cga agg acc acg acg gca tcg gtt tct ggc tac gag      1199
266 Ser Gly Gly Arg Arg Arg Thr Thr Thr Ala Ser Val Ser Gly Tyr Glu
267     210                 215                 220
271 gcg cag acc tac ctc aat ccg ttt ctc acc ggc gag ctg atc ttc gag      1247
272 Ala Gln Thr Tyr Leu Asn Pro Phe Leu Thr Gly Glu Leu Ile Phe Glu
273 225                 230                 235                 240
275 aag taggggactg cacccagatc aggaaacgtc gcggttcatt gttttttttt          1300
276 Lys
278 tttttttttt tttaatgcat gtagagggat atacactaca gtcaagatcg gaattggaga   1360
280 ttagctcata gaaatggtaa ctgcccaaga aacaaaaaaa gaaatgacta acaaatgggc   1420
282 aataataccc tcaatacctt gtcataccta tttgaatgga gaaataactc agttaagctc   1480
284 agtactggca taagcatggg gaaaatattt caattaatca gttcgagtag atatgttttc   1540
286 caaattgata gcgatattag acatttcata ttgaaattta caggtacaaa tataatctca   1600
288 gttaatgcgt agaatgcgtt tgcaattgaa caaatttaac gttttatagc aagaacttag   1660
290 gaacaaaagt tgtaatcgct tattctataa taatgttaac taaagccaaa gcaagtagat   1720
292 cggttgtata actcatttct gctatagata agtcttgact tgtatcagta ctgagctata   1780
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VERIFICATION SUMMARY   DATE: 07/15/2001
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L:13 M:270 C: Current Application Number differs, Replaced Current Application Number
L:15 M:271 C: Current Filing Date differs, Replaced Current Filing Date

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<110>   Pak, William L.
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<120>   Calcium Channel Regulators

<130>   7024-384/PUR-92

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cctgaactca tcggaagtga ccgcggagca gttctacaag cacatcctcg agcagtaccg   300

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Gln Gln Leu Glu Ala Asn Asn Arg Phe Gln Glu Val Phe Ala Thr Ala
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Pro Lys Lys Pro Leu Leu Gly Pro Tyr Ser Pro Gln Pro Gly Asn Ile
        50                  55                  60

agt cac gct atg ggt ggt gat cag ttg gat gca gaa acg gaa cag ggt        767
Ser His Ala Met Gly Gly Asp Gln Leu Asp Ala Glu Thr Glu Gln Gly
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His Met Pro Leu Ile Leu Asp Thr Ser Pro Pro Val Glu Val Thr Gly
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Arg Ala Arg Ala Gly Gly Gln Lys Lys Leu Ser Ile Ala Asn Ser Met
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1/14

$$\frac{w\ pCaspeR}{Y}\ ;\frac{+}{+}\ ;\frac{\pm}{+}\quad X\quad \frac{w}{w}\ ;\frac{\pm}{+}\ ;\frac{Sb\ P[\Delta2\text{-}3]}{TM2}$$

$$\frac{w\ pCaspeR}{w}\ ;\frac{\pm}{+}\ ;\frac{Sb\ P[\Delta2\text{-}3]}{+}\quad X\quad \frac{FM0}{Y}\ ;\frac{\pm}{+}\ ;\frac{\pm}{+}$$

$$\frac{w\ pCaspeR^*}{FM70}\ ;\frac{\pm}{+}\ ;\frac{\pm}{+}\quad X\quad \frac{trol}{W^+Y}\ ;\frac{\pm}{+}\ ;\frac{\pm}{+}$$

$$\frac{w\ pCaspeR^*}{Y}\ ;\frac{\pm}{+}\ ;\frac{\pm}{+}\quad X\quad C(1)\ RM,\ y\ w/Y$$

A: $\dfrac{w\ pCaspeR^*}{trol}\ ;\dfrac{\pm}{+}\ ;\dfrac{\pm}{+}$

B: $\dfrac{FM70}{trol}\ ;\dfrac{\pm}{+}\ ;\dfrac{\pm}{+}$

C: $\dfrac{FM70}{W^+Y}\ ;\dfrac{\pm}{+}\ ;\dfrac{\pm}{+}$

D: $\dfrac{w\ pCaspeR^*}{W^+Y}\ ;\dfrac{\pm}{+}\ ;\dfrac{\pm}{+}$

Stable line:

Males: $\dfrac{w\ pCaspeR^*}{Y}\ ;\dfrac{\pm}{+}\ ;\dfrac{\pm}{+}$

Females: C(1) RM, y w/Y

Fig. 1

09/700869

Fig. 2

3/14

wild type

trp[301]

inaF

| - 1 sec - |

Fig. 3

wild type

trp^{301}

inaF

|– 10 msec –|
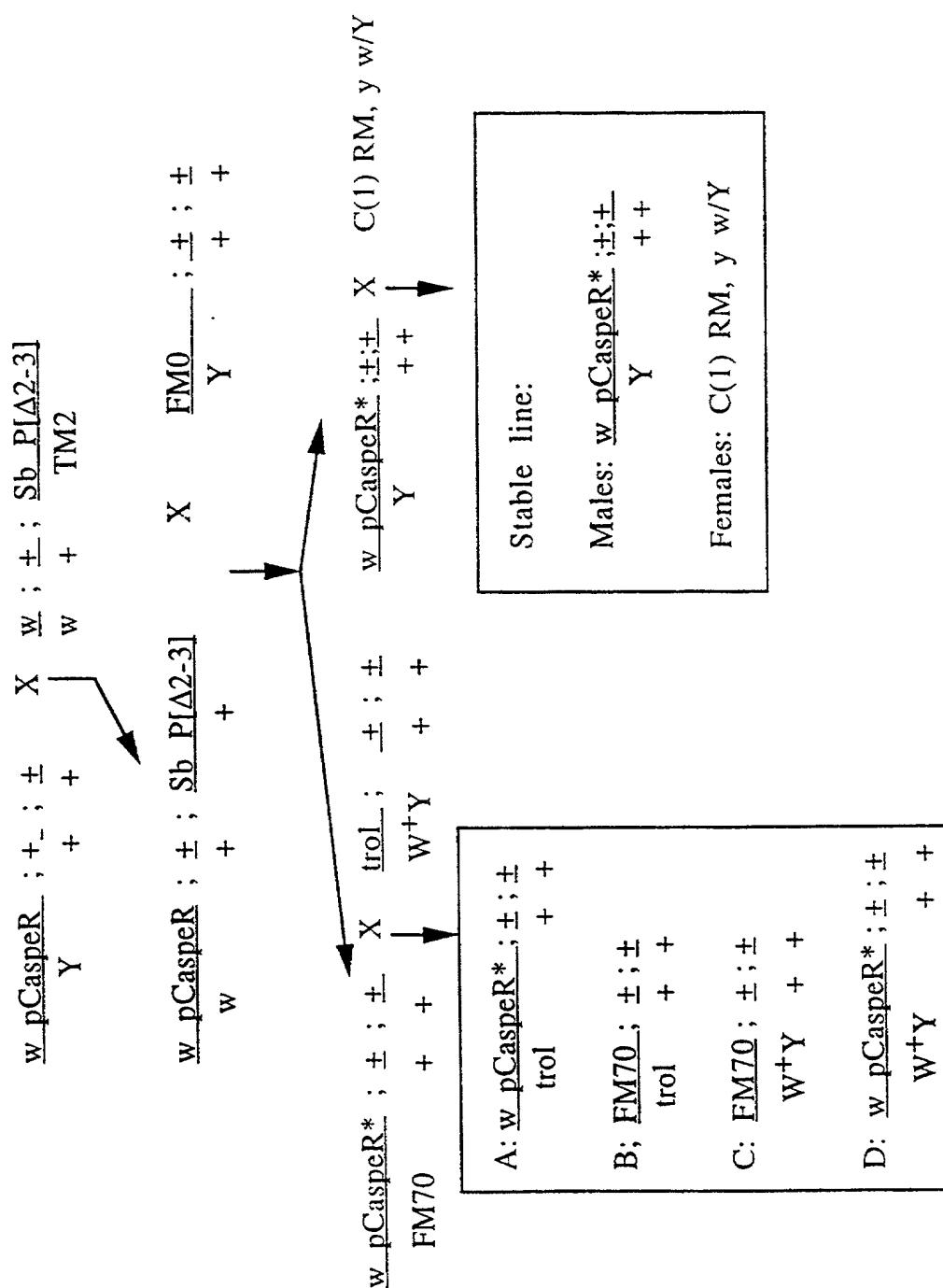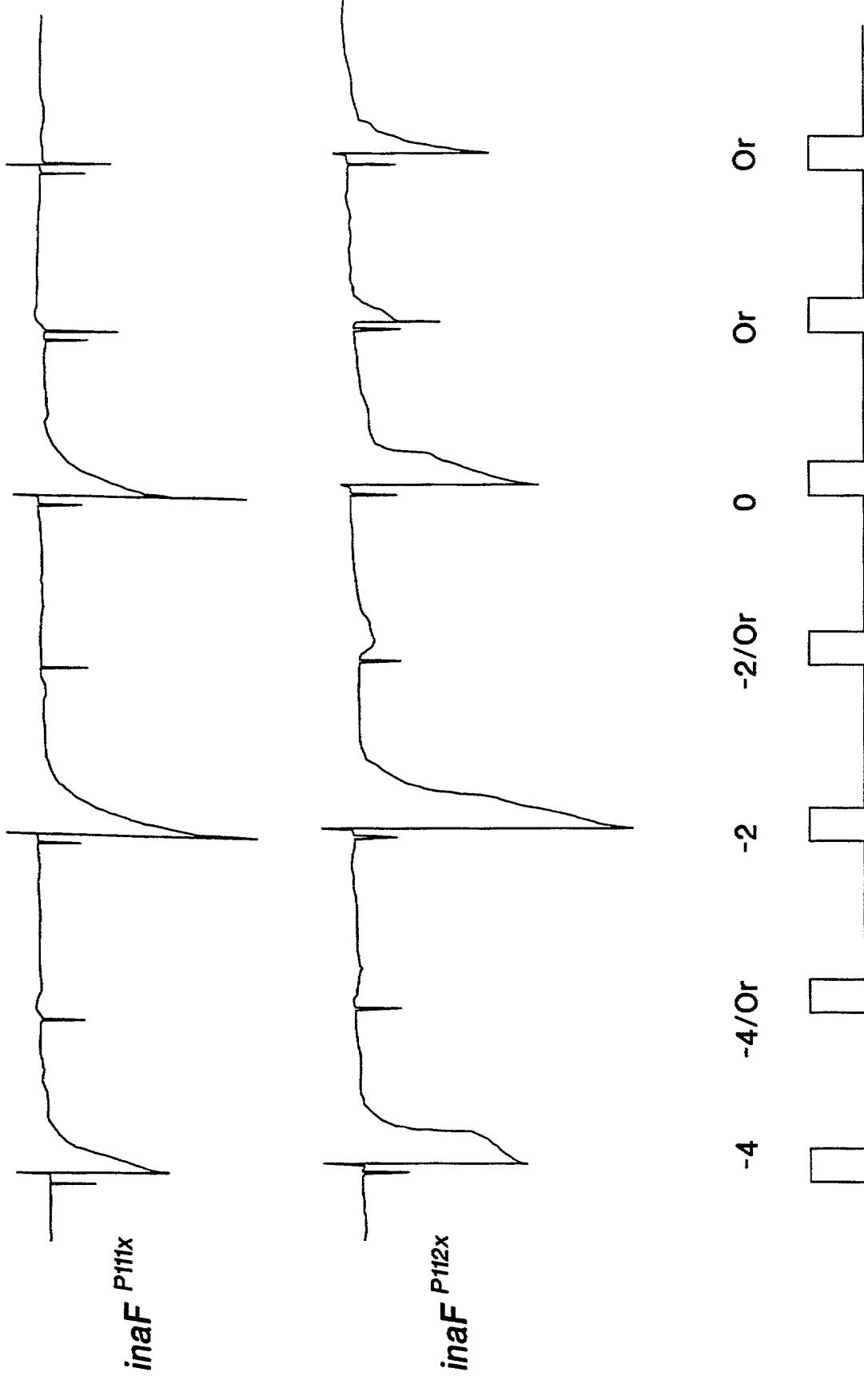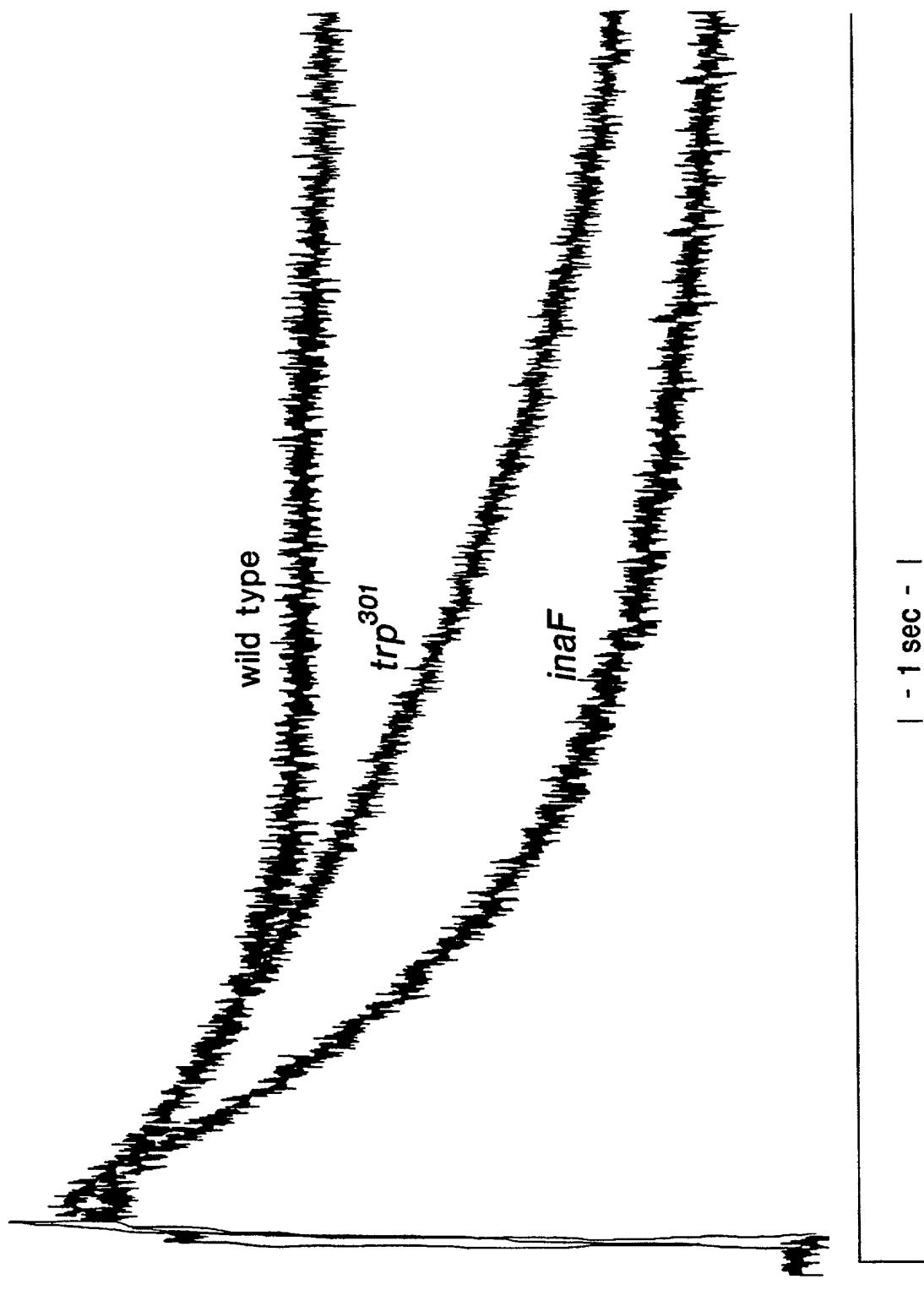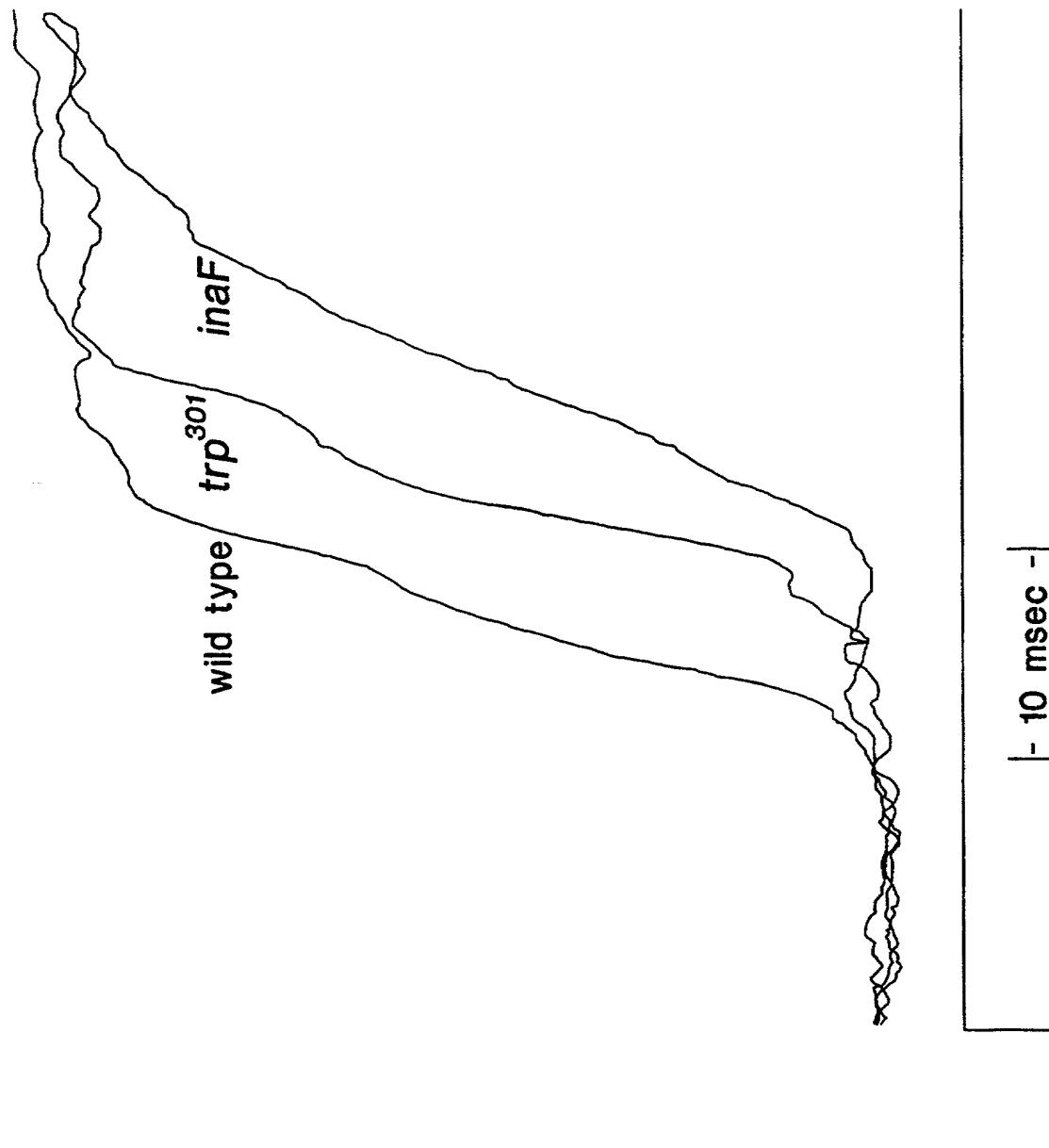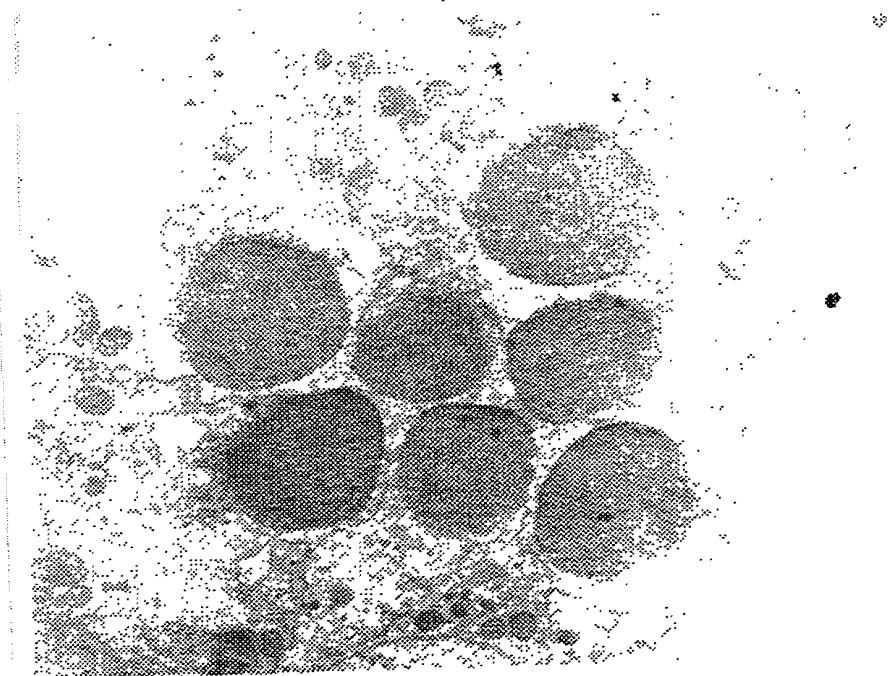
*Fig. 4*

inaF;bw;st 19 days old
Light/Dark reared

Wild Type

Fig. 5A

inaF;bw;st 19 days old
Light/Dark reared

Fig. 5B

inaF ; ± ; ±   X   w ; ± ; Sb   P[delta2-3]
inaF   +   +   Y   +   TM2

inaF ; ± ; Sb   P[delta2-3]   X   Df(1)64C4 ; ± ; ±
Y   +   +   FM0   + +

X-inaF-X ; ± ; ±   X   FM0 ; ± ; ±
FM0   + +   Y   + +

A: X-inaF-X ; ± ; ±
      FM0   + +

B: FM0 ;   ± ; ±
      FM0   + +

C   FM0 ;   ± ; ±
      Y   + +

D.   X-inaF-X ; ± ; ±
      Y   + +
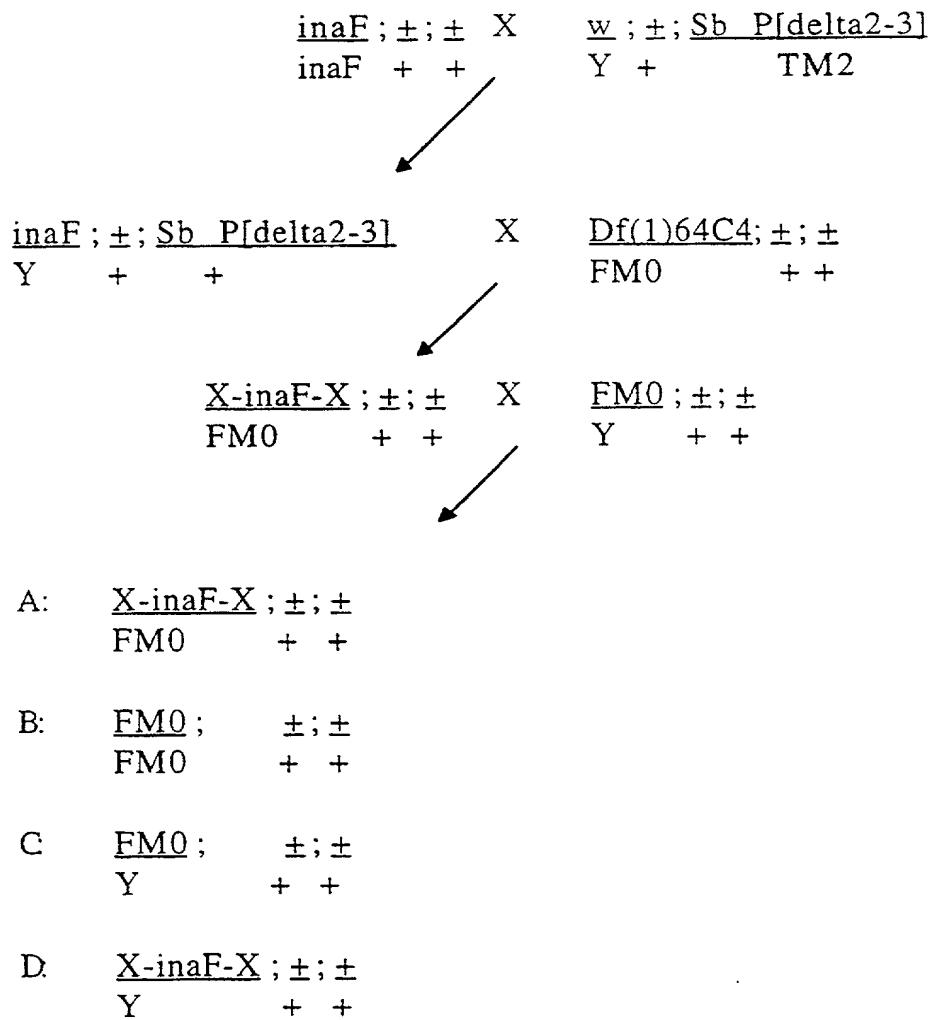
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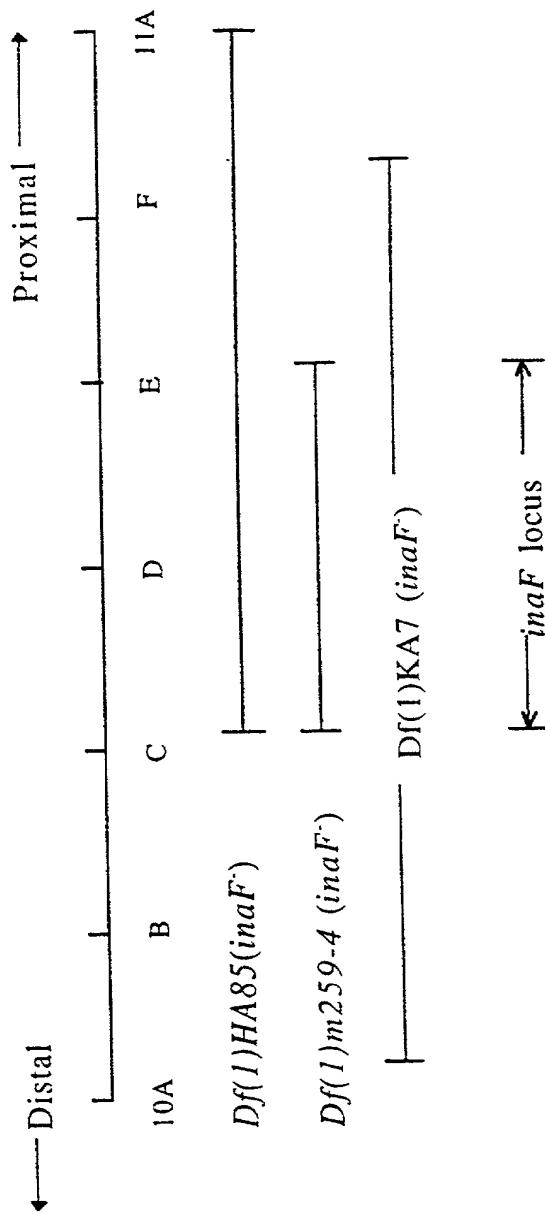
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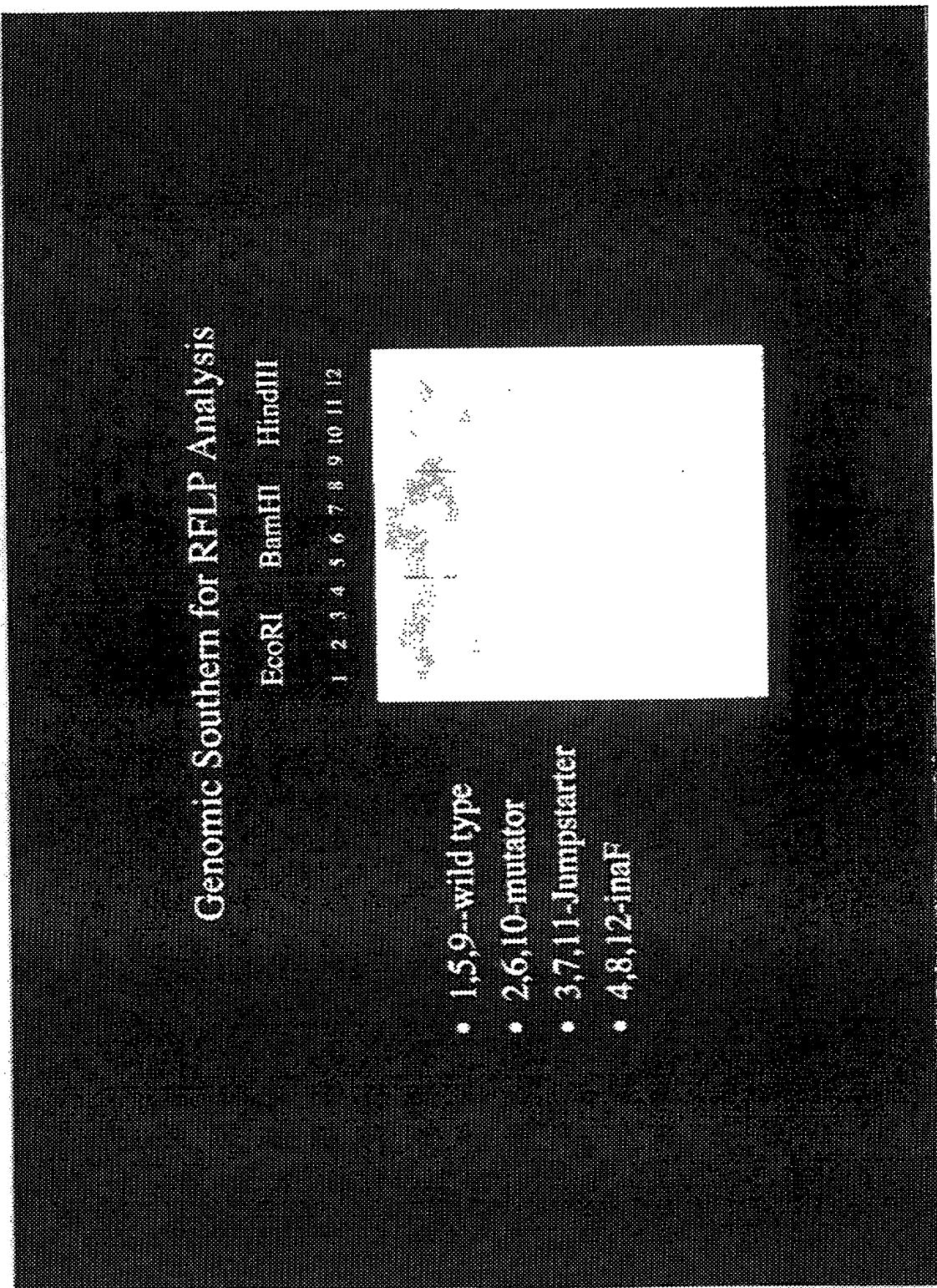
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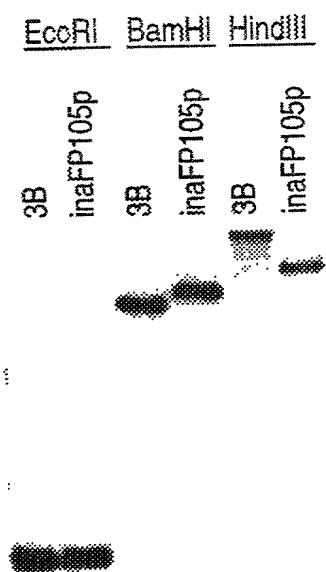
Fig. 9

Fig. 10
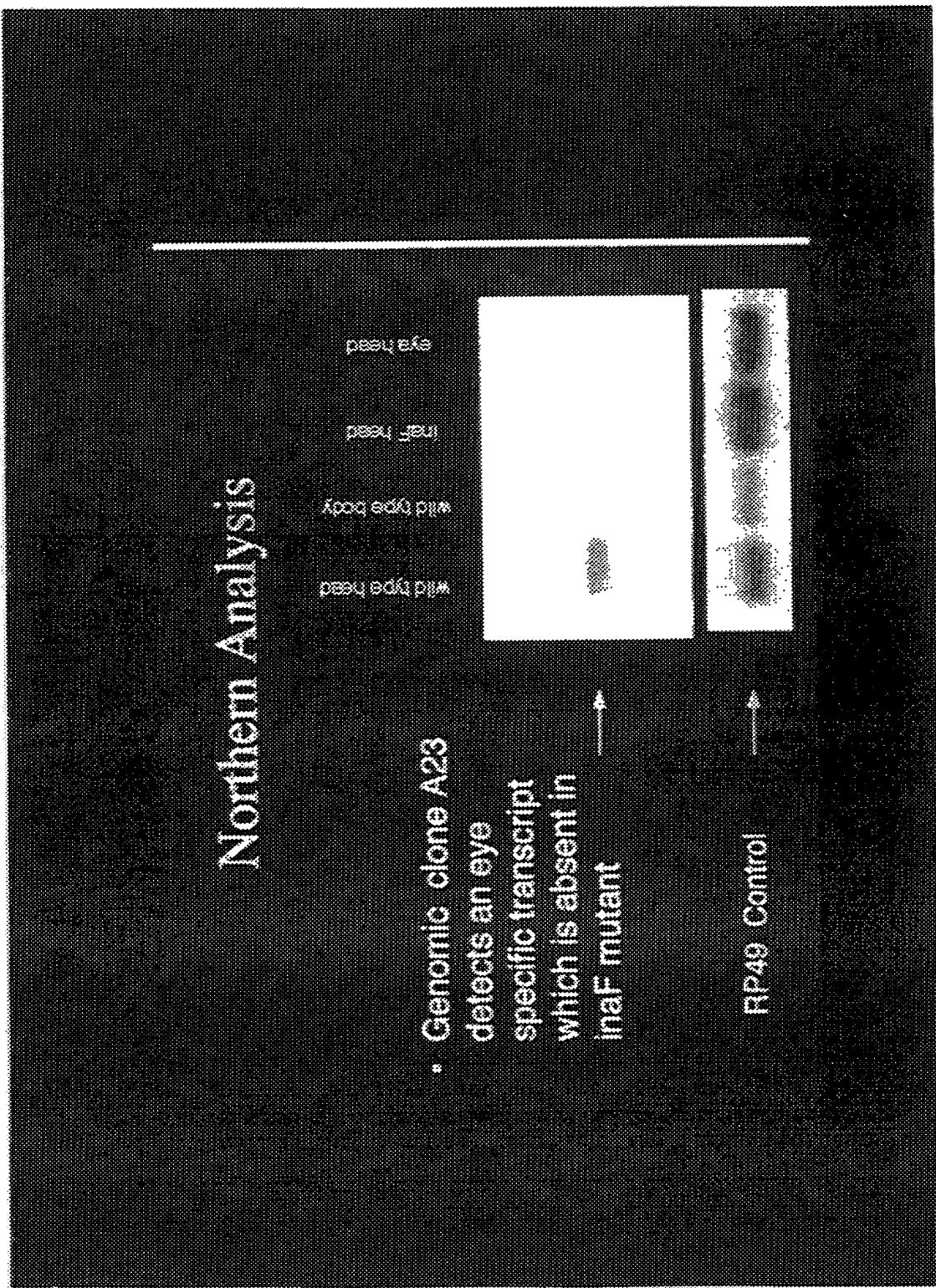
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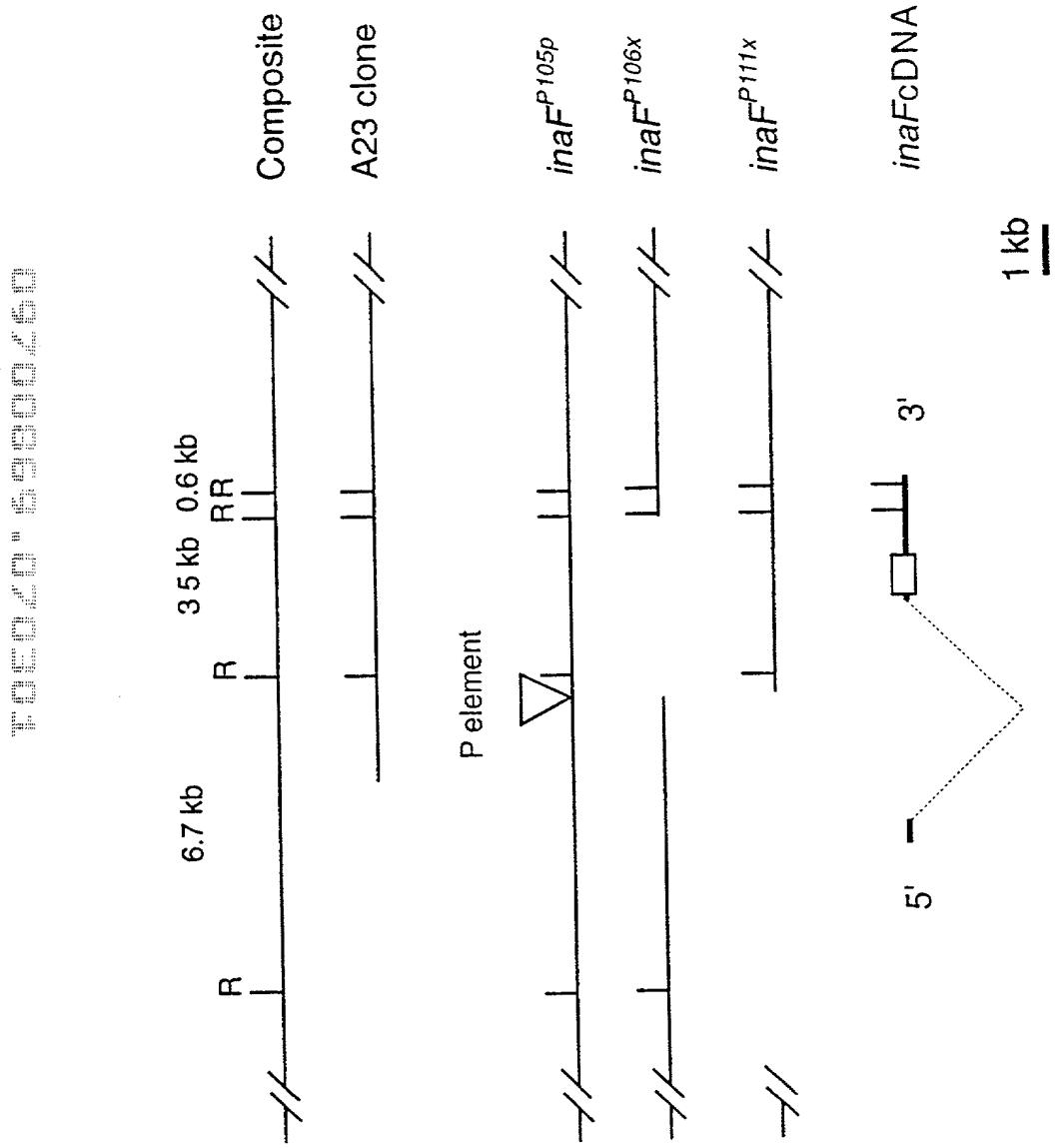
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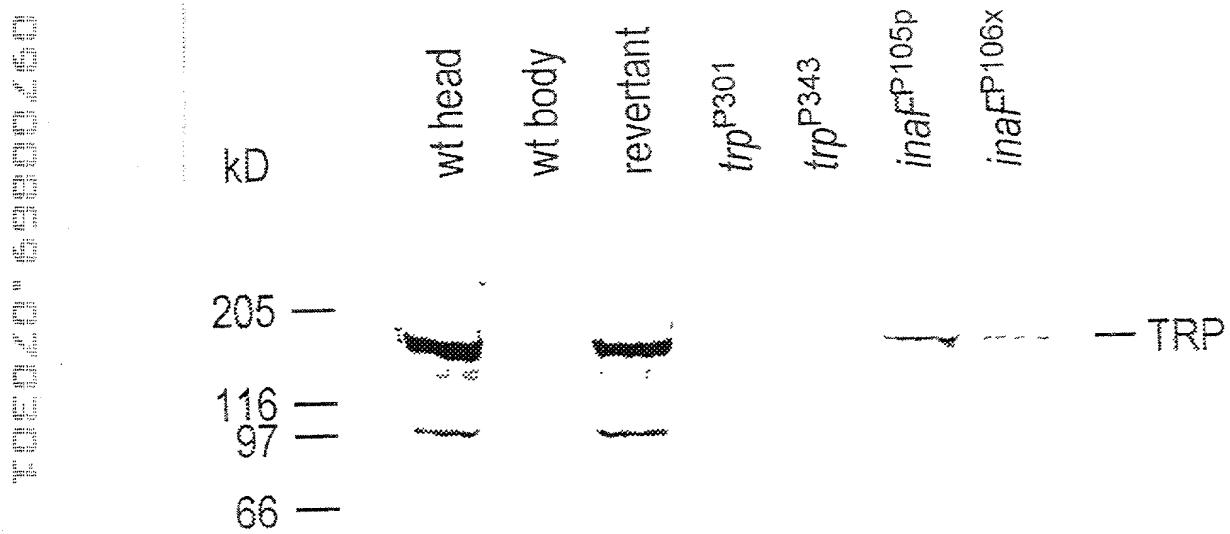
Fig. 13

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JJS:132117:jj

WENMM SB/01 (4-99)

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)	Attorney Docket Number	7024-491
	First Named Inventor	William L. Pak et al.

		COMPLETE IF KNOWN
☐ Declaration Submitted With Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge 37 CFR 1.16 (e) required)	Application Number	09/700,869
	Filing Date	November 20, 2000
	Group Art Unit	Unknown
	Examiner Name	Unknown

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CALCIUM CHANNEL REGULATORS

(Title of the Invention)

the specification of which

☐ is attached hereto   **OR**   [X] was filed on (MM/DD/YYYY) [11/20/2000]   as United States

Application Number [09/700,869] and was amended on (MM/DD/YYYY) [ ]   (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge and hereby disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Check Only If Priority Not Claimed	Certified Copy Attached? YES NO

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
60/087,368	**05/18/1998**	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.
60/098,072	**08/27/1998**	

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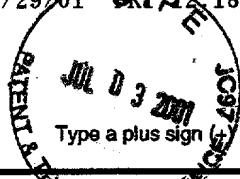
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DECLARATION – Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below, and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

U.S. Parent Application or PCT Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (*if applicable*)
PCT/US99/10821	05/18/1999	

	Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

		Customer Number		Place Customer Number Bar Code Label Here
		OR	→	
X		Registered practioner(s) name/registration number listed below.		

Name	Registration Number	Name	Registration Number
Jason J. Schwartz	43,910		

X	Additional registered practioner(s) named on supplemental Registered Practioner Information sheet PTO/SB/02C attached hereto.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:			A petition has been filed for this unsigned inventor.

Given Name (first and middle [if any])		Family Name or Surname
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| X	Additional inventors are being named on the 2 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

2-w

Name of Joint Inventor, if any:			☐	A petition has been filed for this unsigned inventor.				
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Inventor's Signature						Date	
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Post Office Address							
Post Office Address							
City		State		ZIP		Country	

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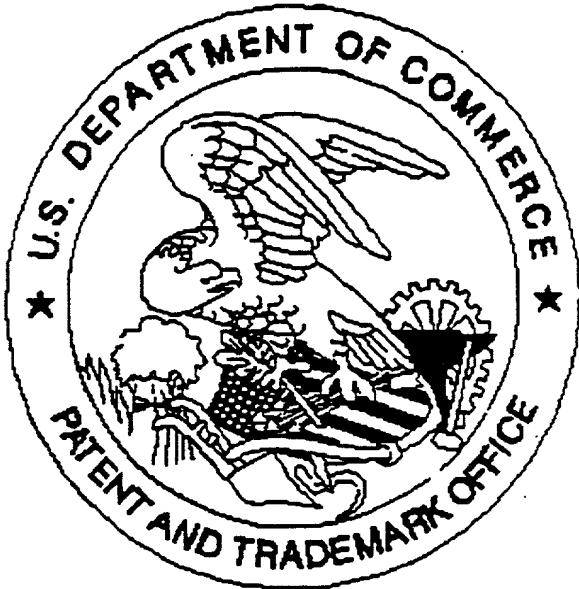
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